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Food Animal Production (101)

Animal Well-Being and Stress Control Systems (105)

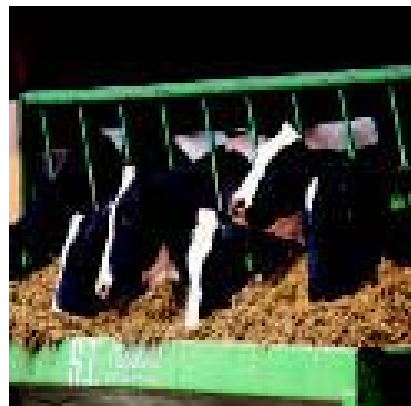
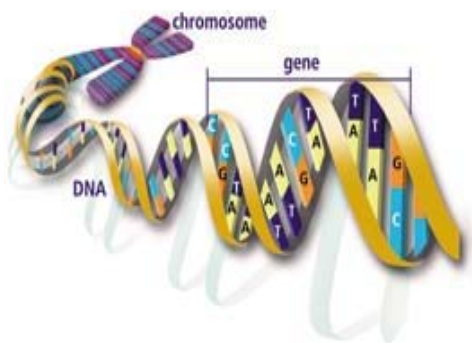


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BACKGROUND AND GENERAL INFORMATION

U.S. systems of agricultural animal management and production face formidable challenges. One of the most exacting challenges is successful adaptation to the accelerating demands of society that affect animal productivity and product quality. The demands placed on the national system of food animal production by a rapidly changing world can only be met by technologies that optimally harness the inherent genetic potential of plant and animal germplasm in concert with industry and food marketing certified practices. Production systems that optimally preserve and harness that genetic potential will maximize profits, secure supply, stabilize prices, increase market competitiveness, and avoid losses from genetic vulnerability and boycott of products.

The Agricultural Research Service (ARS) is the intramural research agency for the U.S. Department of Agriculture (USDA), and is one of four agencies that make up the Research, Education, and Economics mission area of the department. The ARS budget in 2006 of \$1.15B is allocated to research conducted in 22 national program areas. Research is conducted in 108 laboratories spread throughout the United States and overseas by over 2,200 full-time scientists within a total workforce of 8,000 ARS employees. The two ARS national programs addressing animal production are NP 101 (Food Animal Production) and NP 105 (Animal Well-Being and Stress Control Systems). NP 101 involves research conducted at 17 U.S. locations by 91 full-time scientists and has an annual appropriated budget of approximately \$40 M. NP 105 involves research conducted at 4 U.S. locations by 11 full-time scientists and has an annual appropriated budget of approximately \$5 M.

The vision for these two national programs is *“to furnish scientific information about biotechnologies and management practices that ensure an abundant supply of competitively priced animal products”*. Consequently, the overall mission is to safeguard and utilize animal genetic resources, associated genetic and genomic databases, and bioinformatic tools to ensure an abundant, safe, and inexpensive supply of such products from animals produced in acceptable systems for the United States and other nations. This National Program mission follows from the USDA/ARS Strategic Plan (<http://www.ars.usda.gov/aboutus/docs.htm?docid=1766>) which, in turn, is directed towards achieving goals mandated by the USDA Research, Education, and Extension Mission Area Strategic Plan and the USDA Strategic Plan for 2002-2007 (see <http://www.usda.gov/ocfo/usdasp/usdasp.htm>).

The products of research conducted in these two national programs contribute toward broader goals (termed “Actionable Strategies”) associated with four specific Performance Measures from the ARS Strategic Plan for 2003-2007.

Goal 1: Enhance Economic Opportunities for Agricultural Producers.

Performance Measure 1.2.1: Provide producers with scientific information and technology that increase production efficiency, develop improved germplasm, safeguard the environment, improve animal well-being, and reduce production risks and product losses. Baseline: Key animal production systems have been identified and research is being conducted that will lead to more efficient production techniques that safeguard the environment and reduce production risks. Target: Specific information and technology will be available to food animal producers for evaluating animal productivity and well-being, increasing efficiency, and decreasing environmental impact through improved management models and reproduction methods.

Performance Measure 1.2.2: Develop needed information on the relationships between nutrients, reproduction, growth, and conversion to and marketability of animal products. Baseline: Information exists for several economically significant species on the relationship between feed intake, utilization, and nutrient requirements related to animal growth. Target: Information will be

available to producers for more efficiently converting improved knowledge about the interaction of reproduction, growth, and nutrient intake to increase marketability of food animals.

Performance Measure 1.2.3: Identify genes responsible for economically important traits, including animal product quality, efficiency of nutrient utilization, and environmental adaptability. **Baseline:** Identified important quality trait loci in a variety of food animals and made progress on sequencing parts of several animal genomes. **Target:** Better understanding will be available of how genes are responsible for economically important traits in food animals, such as nutrient utilization and environmental adaptability.

Performance Measure 1.2.4: Maintain, characterize, and use genetic resources to optimize and safeguard genetic diversity and promote viable, vigorous animal production systems. **Baseline:** Establish a repository and develop techniques for the long-term preservation and identification of genetic resources of economically significant animals. **Target:** The diversity of food animal germplasm will be maintained and optimized to invigorate production systems.

PLANNING AND COORDINATION FOR NP 101& NP 105

USDA/ARS National Programs follow a five-year program cycle, initiated by a National Program Planning Workshop. Prior to the beginning of the current five-year cycle, a strategy session entitled “FAIR 2002 – Food Animal Integrated Research” was convened in 1999 by the Federation of Animal Science Societies and the Animal Agriculture Coalition in Washington, DC (see <http://www.fass.org/fair2002.pdf>). To respond to the goals outlined by FAIR 2002, a *Joint USDA ARS-CSREES Animal Agriculture Stakeholders Workshop* was held in November of 2001 (see <http://www.ars.usda.gov/docs.htm?docid=1083>).

The first NP 101 National Program Cycle began with a workshop held February 1-3, 2000, in Beltsville, Maryland and for NP 105 with a similar workshop held April 20-21, 1999. ARS scientists and administrators met with customers, stakeholders, and partners and discussed major animal agricultural issues and research priorities. Based on these listening and in-depth discussions, major Research Components for these National Programs were identified, prior to developing the NP 101 & 105 Action Plans (see http://www.ars.usda.gov/research/programs/programs.htm?np_code=301&docid=1013).

The NP 101 Action Plan was drafted by writing teams composed of ARS scientists and members of the USDA/ARS National Program Staff (NPS). The writing teams combined input from the workshops, their own knowledge of the subject matter area, and input from other ARS scientists and their cooperators to identify the key priority needs that could be addressed by ARS research in the coming five year period. These needs were aggregated into Problem Areas for each National Program Research Component. After a public comment period, the draft Action Plan was revised, edited, and completed in 2001. The NP 105 Action Plan was written by the National Program Team in May, 1999 with field review.

Once the Action Plans were completed, ARS project teams each developed a project prospectus containing objectives of specific research from the framework of the Action Plans. Prospectus writing was delayed until 2003 for NP 105 to balance the work load for managing peer-review. Project Plans were then written by project teams of scientists after NPS approval of their project prospectus. Project Plans included statements of the anticipated products or information to be generated by the project, how they contributed to solving the larger National Program Problem Areas, and time lines and milestones for measuring progress toward achieving the project goals. All project plans associated with NPs 101 & 105 were then evaluated for scientific quality by external peer review panels. The project peer reviews were administered by the ARS Office of Scientific Quality Review (OSQR). Project Plans were revised in response to review panel recommendations, and were then approved for implementation. One project plan in NP 101 and one project plan in NP 105 was reviewed by ad hoc panel because of program

increase funding or scientific staff vacancies during the five year period. Project plans were approved for the period of July 2002 to July 2007 in NP 101 and for the period of September 2002 to August 2007 in NP 105.

Five years since the first NP 101 Customer-Stakeholder Workshop in 2000 and the first NP 105 Customer- Stakeholder Workshop in 1999, the progress achieved in attaining the Action Plan goals is now being assessed by an external retrospective assessment panel. In the case of NP 105, research accomplished prior to peer review in 2003 is included in this accomplishment report since the Action Plan was in place to provide the guidance for development of objectives. This program assessment is in preparation for the beginning of the next five-year national program cycle (July 2007-July 2012). **In the next cycle, which will begin with a national program stakeholder workshop in April of 2006, these two programs will be merged in to a single national program called Food Animal Production.**

NPs 101 & 105 are the two ARS National Programs embracing warm-blooded food animal production research and require ongoing coordination at the national level. Such day-to-day coordination is the task of the National Program Leaders who comprise the NPs 101 & 105 Leadership Team. These two national programs are also coordinated with other ARS National Programs and with activities of other agencies. For example, discussions and analyses of the National Science and Technology Council Interagency Working Group on Domestic Animal Genomics and the USDA Task Group on Animal Welfare coordinate and align NP 101 animal genomics and well-being research with efforts in other agencies across the Federal government. These interagency working groups include representatives from the USDA (ARS and CSREES, Cooperative State Research Education and Extension Service), National Science Foundation (NSF), National Institutes of Health (NIH), Department of Energy (DOE), Food and Drug Administration (FDA), U.S. Agency for International Development (USAID), Department of Homeland Security (DHS), Office of Science and Technology Policy (OSTP), and the Office of Management and Budget (OMB).

In addition to ongoing planning and coordination during the first NP 101 Program Cycle 2000-2005, USDA/ARS National Program Staff and Area Offices organized and conducted several workshops focused on specific research issues relevant to the needs of U.S. agriculture. Most of these workshops also involved coordinating and integrating ARS NP 101 efforts with those of cooperating agencies, and with university and industry partners. A partial list of workshops appears in **Appendix 1 – Selected Supporting Information and Documentation for Accomplishments and Impact of NP 101 and NP 105 Research (1)**.

HOW THIS REPORT WAS CONSTRUCTED AND WHAT IT REFLECTS

In this Report, information about NP 101 and 105 achievements and their impact is organized according to National Program Research Components and their constituent Problem Areas, described in the current National Program Action Plan. The report first outlines the eight NP 101 Research Components. This is followed by a section for each of the components. The actual language from the current Action Plan is given to outline the **committed goals, planned approaches, expected outcomes, and engaged ARS locations** for each of the Problem Areas within each Component. These are followed by **selected accomplishments** achieved during the last five years and include the **impact and/or potential anticipated benefits** of those achievements on solving the problems and meeting the high priority needs identified by customer/stakeholders in the NP 101 action plan. The same process is followed in the second section of the report covering the NP 105 action plan and resulting accomplishments and impacts.

For the most part, the content of this report is derived from responses to a recent survey of the NP 101 and 105 scientists who were asked to summarize their project's major accomplishments during the last five years, in terms of impact, and key references documenting those accomplishments. Consequently,

this report does **not** include **all** accomplishments achieved in the national program but, rather, only those **selected** by the ARS scientists polled and the National Program Leaders who authored this report. As a result, the scope of this report encompasses a subset of the total spectrum of NP 101 and 105 accomplishments, chosen to illustrate and exemplify the total progress and achievements **at the national program level**.

Finally, a word about how NP 101 and 105 achievements and accomplishments were documented. Just as only selected accomplishments are reported, the details of those accomplishments are documented selectively so as to illustrate the overall variety of products and knowledge generated by this National Program. In the report text, selected accomplishments found in the narrative are cross-referenced, by numerical citation [e.g., “(1)”, to supporting information presented in **Appendix 1**. Appendix 1 is organized according to the eight NP 101 component areas and six NP 105 component areas.

NPs 101 and 105 encompass 45 appropriated research projects. The titles of the individual projects, objectives, funding levels, and scientific staffing are listed in **Appendix 2 – Listing of Individual Appropriated CRIS Projects by Geographic Location**, which is organized according to the geographical location of the research unit. Each project is coded to reflect the National Program Action Plan components to which it contributes.

Lastly, **Appendix 3 – Annual Report Information (2001-2004)**, provides an aggregated copy of the introduction of the annual reports of the two national programs for fiscal years 2001-2004. Full copies of the annual reports are available on the ARS website (<http://www.ars.usda.gov/research/programs/>), but this information has been excerpted as it provides an overview of new scientists, awards and recognitions, new funding, and additional activities associated with the national programs not reflected by any other section of this report.

National Program 101 – Food Animal Production – Action Plan 2002-2007

Food Animal Production is the ARS national research program that supports scientific needs for delivering a nutritious, high quality, safe and satisfying diet to the American public and people worldwide. Production efficiency and product quality form the basis for profitability and global competitiveness of the U.S. livestock and poultry industry. Increasing efficiency of animal production allows food to be produced with fewer inputs and generating less animal wastes. Greater production efficiency will be necessary to meet demands of the increasing world population, especially if food supplies were threatened. Preserved germplasm enables animal agriculture to respond to changing cultural, regulatory, and ecological environments.

Components and associated problem areas of the action plan include:

Component I: Reproductive Efficiency

- Environmental effects
- Fertile gamete production
- Gamete and embryo storage, sexing, cryopreservation, and use
- Embryo, fetal, and neonatal development and survival
- Interactions of endocrine and immune systems

Component II: Conservation, Characterization, and Use of Genetic Resources

- Characterizing genetic resources
- Preserving genetic resources
- Information systems

Component III: Genetic Improvement

- Develop breeding objectives
- Accelerate selection response
- Improve health and well-being
- Transgenic livestock and poultry

Component IV: Genomic Tools

- Comprehensive maps
- Genotyping systems
- Tools and reagents
- Genomic enhancement systems
- Bioinformatics and statistical analysis tools

Component V: Nutrient Intake and Use

- Regulating gene function
- Interactions affecting reproduction
- Microbial effects
- Minimizing production losses
- Nutrient use and feed evaluation

Component VI: Growth and Development

- Regulating feed intake
- Tissue growth and development

Component VII: Product Quality

- Interactions of genetics and nutrition
- Biological mechanisms controlling variation
- Predicting product quality or defects

Component VIII: Integrated Systems

- User Information Packages

RESEARCH COMPONENT I: REPRODUCTIVE EFFICIENCY

Successful and efficient reproduction is essential to food and fiber production from livestock and poultry. Numerous environmental factors compromise reproductive efficiency and increase unit cost of production. Periods of diminished gonadal activity reduce efficiency or production through added costs from maintaining reproductively inactive animals. Inefficiencies in collection, storage, sexing, and use of semen, oocytes, and embryos limit utilization and conservation of valuable germplasm. Sub-optimal embryonic, fetal, and neonatal development and survival significantly reduce efficiency and profitability. Complex and poorly understood relationships between endocrine, metabolic, and immune systems hinder development and implementation of improved systems for managing reproduction.

Vision Statement: Produce reproductively efficient domestic livestock and poultry that require fewer resources, produce less waste, and supply animal products that more fully meet consumer expectations.

Mission Statement: We seek to mitigate environmental conditions that reduce reproductive efficiency; enhance production of fertile gametes and survival of embryos, fetuses, and neonates; understand interactions between reproductive and immune systems; and ultimately achieve optimum reproductive rate per breeding female.

Impact: Decrease overhead and unit cost of production in all farm animal species, resulting in greater profitability for U.S. livestock producers and in lower food costs for consumers.

Linkages: USDA-ARS National Programs: 103 Animal Health; and 105 Animal Well-Being and Stress Control Systems.

Other Agencies and Universities: USDA-CSREES, Carroll College, Howard University, Purdue University, Texas A&M University, Virginia Tech, Alcorn, Montana, North Dakota, Ohio, Oregon, and Utah State Universities, and Universities of Florida, Georgia, Idaho, Kentucky, Missouri, Nebraska, and Tennessee.

Private sector: National Association of Animal Breeders, Danbred USA, Monsanto Company, U.S. Poultry and Egg Association, Select Sires, Inc., Viagen, Inc.

Problem Area IA -- Environmental Effects

Problem Statement: Reproductive efficiency is affected by numerous environmental factors including temperature, humidity, photoperiod, nutrition, and non-specific stressors. Environmental factors are detected by higher brain centers which affect the neuroendocrine system, subsequent pituitary hormone secretion, and secretion of other hormones. Environmental factors may also directly influence gonadal and uterine function and the conceptus. Managing the environment for optimum reproductive efficiency requires understanding basic neuroendocrine regulatory mechanisms, gonadal and uterine function, and conceptus development. These systems may be further altered by other environmental factors including social interactions among animals, handling by humans, housing, and transportation.

Committed Goals: 1. Elucidate environmental influences on specific components of reproductive performance. 2. Mitigate environmental effects on critical control points limiting reproductive efficiency.

Planned Approaches: 1. Determine how to mitigate the effects of environment on reproductive performance. 2. Determine environmental effects on neuroendocrine pathways controlling adrenal, thyroid, and gonadal function, and behavior. 3. Determine direct environmental effects on fertilization, implantation, embryo survival, pregnancy, parturition, and egg production.

Expected Outcome: Management techniques and production systems that optimize reproductive efficiency by reducing negative environmental effects.

Engaged ARS Locations: Athens, GA; Beltsville, MD; Brooksville, FL; Clay Center, NE; Dubois, ID; Miles City, MT.

Selected Accomplishments:

Heat tolerant breed cattle embryos less affected by heat shock (1). Heat tolerant breeds possess the ability to better regulate rectal temperature but also appear to offer gene or cellular mechanisms to cope with heat stress. A series of collaborative studies conducted with researchers at the University of Florida have demonstrated that embryos (> 5 cells) produced in vitro from heat tolerant cattle breeds (Brahman and Romosinuano) and heat sensitive breeds (Angus and Holstein) both decrease embryo development and cell number when subjected to heat shock. However, the deleterious effect of heat shock on embryo development and cell number was less pronounced for heat tolerant than for heat sensitive breeds. Likewise, lymphocytes from Brahman and Senepol cows experienced less apoptosis (cell death) following heat shock than lymphocytes from Angus and Holstein cows. It is likely that the evolutionary forces that led to breeds being adapted to hot climates resulted in selection of genes controlling resistance to cellular heat shock.

Identification of high sexual performance in rams leads to higher pregnancy rates (2). Scientists at the U.S. Sheep Experiment Station determined that a single three-ram cohort serving capacity test is an efficient and accurate method for separating high sexual-performance from low sexual-performance rams. Previous research indicated that high sexual-performance rams will mate with approximately twice as many ewes during a defined breeding period and sire approximately twice as many lambs as will low sexual-performance rams. Thus, low sexual-performance rams reduce overall flock reproductive efficiency and increase the cost of producing lambs. The results of the cohort serving capacity research indicate that an effective and “producer-friendly” procedure can be used to quickly and accurately identify low sexual-performance rams and remove them from the breeding flock, before they have the opportunity to reduce overall flock reproductive efficiency. Additional studies have determined that early exposure of 6 to 7-mo-old rams to estrual ewes improved their sexual performance scores more than did no exposure or late exposure as adult rams before sexual performance testing. This study indicates that a brief (17 to 21-day), early exposure to estrual ewes is useful for reducing sexual behavior problems of rams. The practice should improve mating performance of young rams and pregnancy rates of ewes mated with young rams in commercial sheep flocks.

Identification of a “latent” form of photorefractoriness for predicting poor reproductive performance in layers (3). A major reason for the short reproductive season (and poor hatching egg production) of the turkey hen is “photorefractoriness” - a failure of the neuroendocrine system to maintain egg production due to a progressive insensitivity to long day length. This decline in photosensitivity is thought to be “programmed” early in the reproductive cycle by long day length and hormone changes. ARS research established that a latent form of photorefractoriness is present in some (but not all) hens early in the reproductive season and demonstrated that a hen’s egg production response to a simple change in photoperiod can identify hens exhibiting this trait. Early expression of this trait seems to provide some prediction of future poor performance. A simple test for latent, or “relative”, photorefractoriness early in the reproductive cycle may be a useful tool to help primary breeders identify

breeding stock that have poor reproductive potential. Additional work is needed to assess whether use of this test for selection would result in improved reproductive performance in future generations, and whether its use is economically feasible when considered with other selection criteria.

“Programming” of photorefractoriness in the turkey hen elucidated (4). A series of ARS experiments determined that the time when programming occurs in the turkey hen is much later than in wild birds. Research results showed that hens will remain photosensitive longer if they are switched from a long photoperiod to a shorter, but still stimulatory, photoperiod by 9 weeks after photostimulation. This is a novel finding, since current industry practice is to maintain or increase day length as the reproductive cycle progresses. These findings suggest that it is possible to provide maximum photostimulation of egg production early in the reproductive season without enhancing the later development of photorefractoriness, and that a subsequent reduction in photoperiod to 12 hours will extend the period of photosensitivity. This work is leading to the development of new light management protocols for turkey producers to extend the lay period and increase hatching egg production.

Cause of Mare Reproductive Loss Syndrome (MRLS) identified (5). With the emergence of an epidemic of reproductive loss in Kentucky mares (2001 to 2003; estimated production losses exceeding \$400 M), the University of Kentucky teamed with ARS to establish the cause of the epidemic and to provide a solution. University scientists in conjunction with the ARS Forage-Animal Production Research Unit identified the Eastern Tent Caterpillar (ETC) via direct feeding studies as the causative agent of the syndrome. As a result, recommendations were made to horse producers to control ETC populations via the use of insecticides and/or removal of cherry trees from their pastures. Although normal population cycles of the caterpillar are likely involved in the decline of MRLS following the 2003 foaling season, these management recommendations are expected to control future ETC population explosions and incidence of MRLS.

Problem Area IB -- Fertile Gamete Production

Problem Statement: Prepubertal development, seasonally reduced gamete production, postpartum anestrus, and aging all represent periods of inefficiency in livestock and poultry. During prepubertal development the hypothalamus and pituitary are highly sensitive to suppressive factors primarily secreted by the gonads. During seasonal declines in gamete production or extended periods of dietary restriction, the reproductive axis is more sensitive to suppressive factors, many of which are produced in the brain or gonad. Opportunities exist to optimize economic returns by determining how to combine genetic and nutritional resources in a manner that reduces the duration of these periods of diminished gonadal activity that result in reproductive quiescence.

Committed Goals: 1. Optimize rate of sexual development and maximize efficiency of gamete production. 2. Lengthen reproductive longevity and minimize periods of gonadal inactivity.

Planned Approaches: 1. Identify genes and gene products whose expression is differentially regulated in individual animals that have superior rates of gamete production. 2. Identify genes and gene products that are associated with enhanced gamete production, sub-optimal photoperiod stimulation, at puberty, following parturition, and at advanced ages.

Expected Outcomes: 1. Understand genetic regulation of pubertal development in livestock and poultry. 2. Increase efficiency of gamete production in aging animals. 3. Technologies to hasten and extend gamete production.

Engaged ARS Locations: Athens, GA; Beltsville, MD; Brooksville, FL; Clay Center, NE; Dubois, ID; Miles City, MT.

Selected Accomplishments:

Genes differentially regulated during puberty identified in swine (1). Profitability of the pork production is dependent on reproductive performance of the breeding herd with one of the leading economic losses due to seasonal infertility. Sixty-three brain, 24 pituitary and 36 ovarian genes that were differentially regulated during pubertal development were identified. A number of these play an important role in activation of the reproductive axis and the onset of puberty. Understanding the interaction of these genes is necessary in order to develop new methods to promote maximal growth and enhance reproductive performance with direct application to seasonal infertility in the sow.

Novel measurements of swine sperm physiology using flow cytometry (2). Boar sperm that have been subjected to long-term low temperature liquid storage (5 days) or freeze-thawing are less fertile after artificial insemination than freshly collected sperm. Physiological changes must occur in the sperm cell (capacitation) before the sperm is capable of fertilization and the acrosome cap on the sperm head must degenerate and release enzymes (acrosome reaction) when it makes contact with the egg in order to penetrate and fertilize. ARS developed rapid in vitro assays and fluorescence-activated flow cytometry methods to identify and quantify different aspects of sperm physiology on an individual viable cell basis. Liquid storage and cryopreservation procedures did not cause significant early maturation of viable boar spermatozoa. These techniques will be valuable in the investigation of basic sperm physiology and to monitor the sperm cell response to experimental changes in hypothermic liquid storage and cryopreservation technology in swine and other species.

Follicle stimulating hormone (FSH) pathways further elucidated in chickens (3). ARS research previously showed that unlike mammals, the chicken produces the two gonad-stimulating hormones (LH and FSH) in separate pituitary cells. ARS scientists now have shown that the brain hormone that regulates LH secretion, chicken gonadotropin-releasing hormone-I (cGnRH-I), does not stimulate secretion of FSH. Additional ARS studies have found that cGnRH-II, a releasing hormone found in all classes of vertebrates but not thought to be a physiological regulator of LH or FSH, significantly increases FSH secretion. These studies will stimulate further research to better understand the regulation of FSH secretion in chickens. This information is of practical importance to poultry producers and scientists, since other studies suggest that early decline in fertility of broiler breeder males is associated with a failure in FSH secretion.

Cattle selected for increased ovulation rate reveal factors affecting recruitment and selection of follicles (4). Because of their extreme level of performance, the ARS U.S. Meat Animal Research Center (MARC) twinning cattle population provides an excellent model for understanding bovine ovarian function, fertile gamete production, uterine function, and their combined effects on fertility. Findings linked increased recruitment and selection of ovarian ovulatory follicles in twinning cows to increased production of insulin-like growth factors and FSH receptors and decreased production of anti-mullerian hormone in twinning cow ovaries. Identification of the regulatory pathways for these factors will enable improved control of ovulation rate and twinning rate in cattle.

Bi-lateral twin ovulations found to enhance the economic value of twinning in cattle (5). Cows expressing twin ovulations gave birth to 0.7 more calves and total weight of calf weaned/cow calving was increased 48% (224.5 vs 331.5 kg/cow for single vs twin births). This large increase in production was partially mitigated by a greater incidence of abortions and dystocia in cows gestating twins. Because embryos do not migrate between uterine horns in cattle, cows expressing bilateral twin ovulations (one ovulation on each ovary) had a lower incidence (14%) of dystocia and bilateral twin calves had a greater survival rate (12%) than cows with both ovulations on the same ovary. Thus, understanding factors controlling bilateral versus unilateral ovulation will allow cattle producers to develop methods to realize the economic benefits of twinning in increased production efficiency. An in vitro ovarian cortical culture system was established from this population to study mechanisms controlling early stages of

development of the female gamete (i.e., oocyte) and of the associated ovarian follicles. When perfected, the system will provide a method for harvesting large numbers of fertile oocytes from a cow, which could be cryopreserved and/or used for in vitro fertilization.

GnRH-induced follicle size delineates fertility differences in beef cattle artificial insemination (AI) systems (6). Reducing embryonic mortality will provide beef producers economically important benefits through increased pregnancy rates and production of calves that are older and heavier at fixed weaning times. Collaborative research between the University of Missouri-Columbia and the ARS Fort Keogh Livestock and Range Research Laboratory, Miles City, MT has revealed that ovulatory follicle size affects fertility of small follicles that are GnRH-induced, but not small follicles that ovulate spontaneously suggesting that in some cases immature follicles are being induced. Direct measures of this decreased fertility appear to be related to reduced estrogen at the time of artificial insemination, which is likely the result of decreased number of granulosa cells. This work indicates that the relationship of GnRH-induced ovulatory follicle size with the fertility of that ovulation may be used as an indicator of fertility from the female without all of the human and male factors affecting fertility measures from individual matings. Continuing work is defining the role of supplemental estrogen in cow AI systems on overcoming lower fertility due to ovulation of immature small follicles.

Estrus synchronization protocols permit implementation of AI without the need for estrus detection (7). In a collaboration with scientists from nine universities and Select Sires Inc., ARS researchers at Miles City, MT synchronized estrus in 2,077 heifers from 12 locations and 2,630 cows from 14 locations to evaluate whether a fixed-time insemination could yield similar pregnancy rates to a protocol requiring detection of estrus, and whether an injection of GnRH at the time of CIDR insertion enhanced fertility. No differences in pregnancy rate among treatments were observed in heifers; however timed AI pregnancy rates among cows that did not receive a CIDR were lower than those receiving a CIDR and those bred following observation of estrus. Pregnancy rates of cows bred following a timed AI only protocol that had received a CIDR were not different than cows bred following observation of estrus. These results indicate that with an appropriate estrus synchronization protocol, timed insemination (without detection of estrus) may be viable for implementation of artificial insemination. This work should lead to greater use of artificial insemination in beef cattle herds.

Problem Area IC -- Gamete and Embryo Storage, Sexing, Cryopreservation, and Use

Problem Statement: Artificial insemination and embryo transfer extend longevity and use of superior germplasm many-fold. However, these technologies are labor intensive and in some species inefficient. New and commercially applicable methods for storage of sperm in liquid or frozen form could greatly enhance reproductive efficiency. New reproductive technologies can further increase the rate of genetic improvement and reduce costs of livestock and poultry production. New technology is also needed to efficiently mature, fertilize, and culture oocytes/embryos *in vitro*. Storage of embryos, oocytes, and somatic cells enables preservation of maternal genetic information and facilitates international trade in germplasm.

Committed Goals: **1.** Develop new and improved existing methods of cryogenic preservation of sperm, somatic cells, oocytes, and embryos for livestock and poultry, placing emphasis on hard to freeze species, breeds, or lines within breeds. **2.** Improve methods for sex-preselection of sperm so that it can be used for conventional artificial insemination. **3.** Improve *in vitro* maturation, fertilization, and culturing of oocytes and *in vivo* developmental competence of mammalian embryos after cryopreservation and embryo transfer.

Planned Approaches: 1. Evaluate biological factors that impact sperm, oocyte and embryo survival after cryopreservation. 2. Develop freezing and thawing methods that improve fertility and survival of cryopreserved gametes and embryos. 3. Develop new technologies to improve reproductive efficiency.

Expected Outcomes: 1. Reliable methods for cryogenic preservation of sperm, oocytes and embryos. 2. Increased reproductive efficiency that expands use of superior germplasm.

Engaged ARS Locations: Beltsville, MD; Brooksville, FL; Fort Collins, CO, Miles City, MT.

Selected Accomplishments:

New methodology explored to allow artificial insemination in sheep (1). Because cervical anatomy limits the use of transcervical intrauterine artificial insemination (TC-AI) in sheep, ARS scientists have developed an instrument to cope atraumatically with the cervix. A series of experiments was conducted to determine whether this instrument affects sperm transport into the oviducts (the site of ovum fertilization), pregnancy rates, or lambing rate. Direct intrauterine deposition of spermatozoa with the new instrument, using a surgical procedure to bypass the cervix, improved pregnancy rate, but, even though the new instrument did not affect sperm transport into the oviducts after TC-AI, the transcervical procedure reduced pregnancy and lambing rates. The TC AI procedure with the new instrument has all but eliminated any visual evidence of cervical trauma during TC-AI, so additional research will be conducted to determine how cervical manipulation associated with the TC-AI procedure reduces pregnancy and lambing rates.

A new method for collecting semen from rams and other animals (2). A vial was designed to fit safely inside of a ewe's vagina and permit a ram to ejaculate into the vial. The vial containing semen can be easily recovered, and the semen can then be extended, frozen, transported, and used to artificially inseminate large numbers of ewes. The technology has been transferred to sheep breeders in Iowa, Nebraska, Oregon, Utah, Virginia, West Virginia, Wisconsin, Canada, and France and deer breeders in Texas, and the device is being used at the ARS U.S. Meat Animal Research Center to collect semen for preservation and addition to the National Animal Germplasm Program, Ft. Collins, CO.

Progeny of cloned boars evaluated (3). At the request of the Food and Drug Administration, MARC scientists undertook an experiment to determine whether there were any inherited effects of cloning on the growth and development of subsequent progeny from cloned boars. Blood and urine samples were collected at intervals from birth to slaughter from the progeny of cloned and control boars and then at slaughter, meat composition traits were also measured. All the required samples have been collected and analysis of samples by ViaGen, Inc. is still ongoing. Results of experiments such as these will be required to support decisions on the use of meat from cloned animals for human consumption.

Physiological impact of lipid peroxidation on turkey semen identified (4). In the U.S., the commercial turkey industry relies exclusively on AI for production of fertile eggs. Given that each breeder hen must be inseminated weekly during a 6 mo egg production period, AI is both time and labor-intensive. Turkey producers would benefit if freshly collected semen could be stored for 24 to 48 hr without affecting fertility. With current technology, the maximum amount of time that turkey semen can be stored without a significant drop in fertility over the course of egg production is only 6 hr. Because turkey spermatozoa utilize aerobic metabolism, most storage protocols involve constant aeration of the semen. ARS research identified a major limitation of conventional turkey semen storage protocols in that liquid storage with constant aeration promotes formation of toxic lipid peroxides that adversely affect fertility rates. The significance of this discovery is directly related to the industry practice of randomly pooling semen from several males for insemination, in that semen from just one tom producing large amounts of peroxides during storage could greatly diminish the fertility of the entire semen pool. As a

result of the research, a major U.S. manufacturer of poultry semen extender (Gobbler's Inc.) has opted not to market antioxidant-supplemented formulations for long-term liquid storage of turkey semen. This lipid research also has led to the development of a potentially patentable new technique to improve the fertility of 24 hr-stored turkey semen, and at least two manuscripts pending review of the Invention Report.

Chicken genome sequence exploited to identify candidate genes for improving turkey semen storage (5). The commercial turkey industry relies exclusively on artificial insemination (AI) for fertile egg production. Due to low success of cryopreserving or storing turkey semen longer than 6 hr, AI procedures rely primarily on the use of fresh semen, necessitating the costly maintenance of male breeder flocks. A unique aspect of turkeys is that they have the ability to store sperm in the oviduct of the hen for extended periods of time in sperm storage tubules (SST). ARS scientists applied serial analysis of gene expression (SAGE) to characterize gene expression of turkey SST-containing mucosa, and found that over 200 genes were differentially expressed within the mucosa depending upon whether or not sperm were present in the SST. The recent release of the chicken genome sequence has been exploited to apply proteomics techniques to identify 40 proteins that are expressed in the turkey SST epithelium. Four proteins were uniquely expressed when sperm were absent while three additional proteins were only expressed when sperm were present. Using a complementary candidate gene approach, the presence of a class of molecules, aquaporins, which regulate water transport across cells in the turkey epididymis (known site of fluid resorption) and SST in the hen was identified. The aquaporins may function in the release mechanism of sperm from the SST. Procedures using whole mounts of surface mucosa for immunocytochemistry expedited the localization of aquaporins in the SST epithelium, and will facilitate functional analyses of gene candidates identified via SAGE and proteomics analyses. New techniques were developed to localize various proteins (alkaline phosphatase, serotonin) in the oviduct's surface epithelium that may influence sperm selection and transport. The fundamental knowledge gleaned regarding oviductal sperm selection, transport and storage will provide a basis for developing novel approaches to *in vitro* semen storage and AI techniques. This is the first reported application of SAGE and proteomics technologies in poultry and these basic findings are facilitating identification of novel gene transcripts and proteins required for maintenance of poultry sperm viability. Having chicken cDNA and genomic sequence data available was critical for successfully applying SAGE and proteomic analyses in the turkey, and thus, these results provide one of the first examples of the power of now having whole genome sequence available from the chicken.

Problem Area ID -- Embryo, Fetal, and Neonatal Development and Survival

Problem Statement: Delays in establishing pregnancy increase cost and reduce output of food animal systems. Maximum production efficiency requires every fertilized egg to result in birth of a healthy offspring that survives and grows during the neonatal period. Factors contributing to embryonic and fetal losses and/or inappropriate development in domestic livestock are numerous and interacting. Incidence of embryonic and fetal mortality has been estimated to be 20 to 40% in livestock species and 10 to 14% in poultry. Others are born with extreme birth weights or other developmental abnormalities that contribute to loss during the neonatal period.

Committed Goals: 1. Prevent abnormal and inefficient embryonic, fetal, and neonatal growth and development in livestock and poultry species. 2. Mitigate abnormal development and growth of embryo, fetus, and neonate. 3. Improve embryonic, fetal, and neonatal survival and health.

Planned Approaches: 1. Identify physiological mechanisms causing inappropriate development and loss in domestic livestock species during the embryonic, fetal and neonatal periods. 2. Develop methods to control physiological mechanisms resulting in inappropriate development and loss and to assess other consequences of these changes. 3. Modify, as needed, those procedures controlling physiological

mechanisms resulting in inappropriate development and loss and disseminate appropriate technologies to the livestock industry.

Expected Outcomes: 1. Enhanced embryonic, fetal, and neonatal development and survival through modification of physiological mechanisms, and genetic selection. 2. Disseminate methodology to improve development of embryos, fetuses, and neonates. 3. Reduce inputs used to obtain healthy offspring.

Engaged ARS Locations: Athens, GA; Beltsville, MD; Clay Center, NE; Dubois, ID; Miles City, MT.

Selected Accomplishments:

Rate of progesterone increase in early pregnancy affects uterine capacity in swine (1). Previous ARS research indicated that increased progesterone on day 2 and 3 of pregnancy accelerated both uterine protein secretion and conceptus development in swine. Recent research determined that progesterone treatment decreased uterine capacity. Subsequently it was determined that decreasing progesterone by treating gilts with the progesterone antagonist RU486 during early pregnancy decreased uterine protein secretion and conceptus development. Finally, contrary to expectations, treatment with RU486 during early pregnancy also decreased uterine capacity. These results indicate that there is likely to be an optimum rate of progesterone increase during the first 2 to 3 days of pregnancy, and that departure from this optimum decreases uterine capacity and litter size. Continuing work on factors determining the rate of rise of progesterone during early pregnancy could provide methods to increase uterine capacity and litter size to allow enhanced reproductive efficiency in swine production.

Characterization of uterine proteins during early pregnancy reveals potential role of glycolipids on pregnancy (2). Germplasm characterization of Meishan swine earlier revealed that slower conceptus growth, decreased uterine protein content, and increased uterine capacity were associated; however, other ARS work indicated that the relationship between uterine protein secretion and uterine capacity was not simply a direct negative relationship. To gain more information on the role of the proteins present in the intrauterine environment during early pregnancy, proteins present in uterine flushings on days 10 and 13 of the cycle and pregnancy were compared using 2D-PAGE, and then were identified using mass spectrometry. Approximately 100 protein spots, corresponding to ~40 genes, were identified. Many of the proteins identified were glycolipid metabolizing proteins whose role during early pregnancy has never been investigated. These experiments provided clues to previously unidentified metabolic pathways within the intrauterine environment that may influence embryo development, uterine capacity and litter size.

Erythropoietin receptor polymorphism found to affect swine litter size (3). ARS research has confirmed a relationship between fetal size and fetal hematocrit, which when combined with additional results indicating accelerated fetal erythropoiesis during early pregnancy in Meishans and increased hematocrits in fetuses from gilts from a line of pigs selected for uterine capacity, suggested an association between erythropoiesis and uterine capacity. Erythropoiesis is influenced by both erythropoietin and by available folate, thus cDNA for erythropoietin receptor (EPOR) and the genes for the secreted form of folate binding protein (sFBP) were developed. MARC scientists have now explored the genes for the erythropoietin receptor and sFBP for genetic polymorphisms associated with uterine capacity and litter size. Polymorphisms associated with uterine capacity were discovered for both genes. However, only the EPOR polymorphism was associated with litter size because the sFBP polymorphism was also associated with decreased ovulation rate. Recent results indicate that the presence of the polymorphism in fetuses is associated with a 10 to 20% increase in EPOR mRNA expression on day 30

of pregnancy. This genetic marker is now available to the swine industry for further testing of its effects on litter size.

Uterine folate-binding proteins affinity explored for effect on uterine capacity in swine (4). ARS scientists investigated the interaction of the two forms of FBP during pregnancy with regard to folate transport, in work funded by a USDA-NRI grant. Using immunohistochemistry, it was demonstrated that sFBP is secreted during early pregnancy by the uterine glands but secretion ends by day 35 of pregnancy. Using folate binding analysis, placental membrane folate binding was undetectable on day 20, increased steadily to day 50 of gestation and subsequently leveled. The affinity of the membranes for folate decreased sharply from day 70 to day 90 of gestation. In situ hybridization analysis confirmed that mFBP is transcribed primarily by the pig placenta during gestation, and suggests that mFBP is at least partially responsible for folate binding by placental microsomal membranes. These results indicate that folate transport to the developing conceptus occurs sequentially, first by sFBP, then by placental mFBP. Thus, the overlapping period, day 20 to 35, when the placenta is forming, represents a window during pregnancy when folate transport may be inadequate, and thus contribute to the substantial fetal loss that occurs during this period, particularly in a crowded intrauterine environment. In addition, folate is unlikely to be the only substrate for which transport occurs in such a sequential fashion, and this suggests further mechanisms for fetal losses during this period. Because approximately 2/3 of the fetal loss due to intrauterine crowding occur during this period, determination of the factors responsible could have a profound effect on uterine capacity and litter size.

Functional genomic analysis of pig embryo development (5). Pig embryos are highly susceptible to early death between gestational days 11 (D11) and 12 (D12), and a critical step in developing new strategies for reducing this embryonic mortality is to identify the genes that are switched on and off during this interval in development. ARS scientists applied a new functional genomics technique, Serial Analysis of Gene Expression (SAGE), to investigate fluctuations in gene expression during the D11 to D12 developmental interval in pig embryos. This work led to the simultaneous discovery and identification of over 430 genes that are expressed at significantly different levels between D11 and D12 stage porcine embryos. SAGE databases generated from these D11 and D12 embryo libraries were transferred to the public domain via deposition with the National Center for Biotechnology Information (NCBI). Analysis of differentially expressed genes in these libraries revealed that estrogen synthesis and metabolism, and oxidative stress response pathways appear to play critical roles in embryo elongation. This is a major advance scientifically that will now enable construction and analysis of SAGE libraries that represent all pre-implantation stages of swine embryo development for the first time. These combined advances demonstrate that SAGE enables simultaneous gene expression analyses at both the metabolic pathway and single gene levels, in embryos from any stage of development, and is thus ideally suited for establishing a novel systems biology approach to investigating swine embryo development. These results are of value for future research that may lead to determination of genetic markers of embryo survival, with a potential increase in litter size, and improve conditions of culture for improvement of in vitro embryo production required to development of embryo biotechnologies.

Problem Area IE -- Interactions of Endocrine and Immune Systems

Problem Statement: Complex interactions between immune and endocrine systems affect many physiological processes, including reproduction. Resolving basic mechanisms that control the many interactions between immune and endocrine systems is essential for improving growth and development, reproductive management, and production efficiency.

Committed Goals: 1. Elucidate the role of the immune system in modulating reproductive activity, mammary function, gametic production and survival, luteal function, pregnancy, uterine involution, and

embryonic development in the female reproductive tract. **2.** Improve uterine immune functions to reduce uterine and oviductal infections without using antibiotics.

Planned Approaches: **1.** Determine cellular and molecular mechanisms by which endocrine secretions regulate uterine immune functions allowing the uterus to eliminate or manage bacterial contamination. **2.** Define cellular and molecular interactions affecting control of reproductive health and efficiency by endocrine and immune systems to influence the reproductive efficiency of both healthy and immune stressed animals. **3.** Determine mechanisms of the immune system that regulate luteal function and gametic development, maturation, and survival in the female reproductive tract.

Expected Outcomes: **1.** New, effective methods for managing reproductive events. **2.** Novel methods for preventing or treating uterine infection and bacterial contamination that do not rely on antibiotics. **3.** Nutritional, genetic, environmental, and management strategies to promote immune functions that enhance reproductive efficiency.

Engaged ARS Locations: Athens, GA; Beltsville, MD; Brooksville, FL; Clay Center, NE; Dubois, ID.

Selected Accomplishments:

Reducing the incidence of uterine infections in sheep (1). Scientists at the U.S. Sheep Experiment Station used intrauterine inoculations with *Arcanobacterium pyogenes* and *E. coli* to evoke an antibody response in nulliparous ewes and then determined that this treatment reduced the severity of subsequent uterine infections. Nonspecific uterine infections reduce the reproductive efficiency of livestock, and uterine infections seem more severe in nulliparous animals. Thus, an experiment was conducted to determine whether intrauterine inoculations could be used to help ewes prevent or reduce the severity of nonspecific uterine infections. The data from this study indicate that it should be possible to develop an efficacious inoculation protocol for nulliparous livestock to reduce the incidence of uterine infections and improve lifetime reproductive performance.

RESEARCH COMPONENT II: CONSERVATION, CHARACTERIZATION, AND USE OF GENETIC RESOURCES

To meet preferences and needs of consumers for animal products, livestock and poultry are produced in a wide array of environments and management systems. Insufficient quantitative and genomic characterization of existing resources compromises efficiency of production across eco-regions of the U.S. and limits optimal use of feed resources and response to diseases. Losses in production efficiency from genotype by environment interactions and gene by gene interactions must be better understood to respond to these challenges and increase profitability. Existing genetic resources provide producers with numerous options that can be tailored to meet future demands. However, several of these resources are at risk of being lost, even before they are adequately characterized. Emerging conservation efforts require a wide range of information and analytical tools.

Vision Statement: Increase competitiveness of livestock and poultry industries through characterization and conservation of genetic resources.

Mission Statement: Improve production efficiency and quality of livestock and poultry products and to conserve genetic resources at risk of being lost.

Impact: Increase profitability for U. S. livestock producers in diverse production environments, and reduce costs and increase quality of food for consumers. Enhance response to changes in consumer preferences and societal standards.

Linkages: USDA-ARS National Programs: 103 Animal Health; 104 Veterinary, Medical, and Urban Entomology; 108 Food Safety.

Other Agencies and Departments: USDA-CSREES, APHIS, Foreign Agriculture Service, The Consultative Group on International Agricultural Research, FAO, U.S. Fish and Wildlife Service, Colorado State University, Michigan State University, Montana State University, Purdue University, Virginia Tech, and the Universities of California-Davis, Florida, Missouri, and Nebraska.

Private Sector: ESVA Agisternoia Veterinaria, American Minor Breed Conservancy, Gensel Biotechnology, Inc., University of Guelph, Livestock Breed Associations, U.S. Beef Breeds Council, and World Bank.

Problem Area IIA – Characterizing Genetic Resources

Problem Statement: Defining characteristics within and between breeds (strain, line) and between species in multiple production environments is a key element to efficiently and profitably meet consumer demand for livestock and poultry products. Effects of individual genes and gene combinations need to be related to animal performance across production systems and in response to disease challenge.

Committed Goals: 1. Quantify genetic variation and genetic distance between and within breeds or strains. 2. Identify phenotypic and molecular differences between breeds and strains for economically important traits. 3. Determine how breed performance is altered by changes in environment or management system.

Planned Approaches: 1. Sample and characterize *in situ* livestock and poultry genetic resources by quantitative analysis and molecular experimentation. 2. Evaluate breeds and strains for economically relevant traits including disease resistance, environmental tolerance, and adaptation to production systems. 3. Elucidate gene function and interactions among genes, identify novel genes, and

characterize allelic variation. **4.** Use point mutation and molecular evaluation technologies to generate new DNA sequences with novel functions.

Expected Outcomes: **1.** Increase profitability through increased understanding of genetic characteristics at the phenotypic and molecular levels. **2.** Recommend to industry how to use diverse genetic resources in diverse production systems and environments and maintain genetic diversity.

Engaged ARS Locations: Beltsville, MD; Brooksville, FL; Clay Center, NE; Dubois, ID; East Lansing, MI; El Reno, OK; Fort Collins, CO; Miles City, MT.

Selected Accomplishments:

Understanding the status of genetic diversity within a breed is necessary to quantify changes in genetic diversity and to develop sampling protocols for germplasm acquisition (1). Computing a breed's inbreeding level is an inexpensive snap-shot of a breed's genetic diversity. Therefore, inbreeding trends were calculated for the following breeds: Navajo Churro (1.2%), Hog Island (2.8%), and Jacob sheep (2.3%), Salers (3.4%), Hereford (10.2%), and Red Angus cattle (3.8%), Yorkshire (5.7%), Duroc (1.9%), Hampshire (5.2%), and Hereford pigs (3.3%) and this information was disseminated to the respective associations. This information serves as a baseline for future comparison and can be utilized by breed associations as they implement programs to minimize inbreeding rates.

Characterization of *Bos taurus* beef breeds (2). In cycle VII of the Germplasm Evaluation Program at the U.S. Meat Animal Research Center (MARC) three breeds of British origin (Angus, Hereford, and Red Angus) and four breeds of Continental European origin (Simmental, Gelbvieh, Limousin, and Charolais) are being evaluated. According to registrations reported by breed associations, these breeds are the seven most prominent breeds used for beef production in the United States. Major findings to date include:

Angus and Red Angus sired calves require less assistance at calving than those by other British or Continental European sire breeds. Even so, calving ease has improved significantly for calves sired by Continental European breeds, especially Simmental and Gelbvieh, relative to those by British breeds since the Continental European breeds were first introduced into the U.S. Sire breed of calf differences for birth weight between Continental European and British breeds are less than half as great in recent years as they were 30 years earlier. Sire breed of dam differences for calving ease and birth weight for 2-year-old first calf heifers progeny are not significant today, reflecting significant improvement in calving ease for Continental breeds relative to British breeds over a 30 year-period. These results, consistent with genetic trends for expected progeny differences (EPDs) for birth weight and calving ease reported by breed associations, indicate that Continental European breeds have emphasized selection for components of calving ease relatively more than British breeds during this time span.

Today, progeny of British and Continental European breeds do not differ in postweaning average daily gain or live weights at yearling ages. In evaluations conducted 30 years earlier, Continental European breeds had significantly faster average daily gains and were 1 – 3 standard deviations heavier at yearling ages than British breeds. These results, also consistent with genetic trends reported by breed association for these seven breeds, indicate that growth rate to weaning and yearling ages has been emphasized far more in British breeds than in Continental European breeds during the past 30 years.

As a result of these relative changes in growth rate, British and Continental European breeds no longer differ significantly in efficiency of postweaning live weight gain (gain, kg/ Mcal metabolizable

energy consumed) to age or weight end-points. However, to fatness endpoints (1.1 cm fat thickness, 25% carcass fat trim, or small00 marbling), progeny by British sire breeds are still significantly more efficient than those by Continental European sire breeds. To weight of retail product endpoints, (i.e., 500 lb), progeny by Continental European sire breeds were still significantly more efficient.

Although, British and Continental European breeds do not differ significantly in live weight at the same age, Continental European sire breeds still produced progeny with significantly heavier retail product (15 kg) due to higher retail product yields (3.9%) at the same age (445 days) than British sire breeds. Steers by British sire breeds had significantly greater marbling and a higher percentage of carcasses grading USDA Choice than those by Continental European sire breeds. Results indicate that differences between Continental European and British breeds for carcass composition, marbling, and tenderness remain nearly as great today as they were 30 years ago, a result that is not surprising because EPDs for carcass traits have only been introduced for most breeds in recent years.

Breeds that have had a long history of selection for milk production (e.g., Simmental and Gelbvieh), still produce females which reach puberty at younger ages than those that have not been selected for milk production (Limousin, Charolais, Hereford, Angus or Red Angus).

Differences among sire breeds for lifetime reproduction rates of F_1 cross daughters were not significant in recent or earlier evaluations.

Weaning weights of progeny of F_1 daughters are still greater for Simmental and Gelbvieh which have a history of selection for milk production than for those by other breeds (Charolais, Limousin, Angus, Red Angus, Hereford); however, contrasts between Continental European and British breeds are less than half as great in recent evaluations than 30 years earlier for direct and maternal components of weaning weight.

Recent estimates indicate that Continental European and British breeds do not differ significantly in estimates of cow weight or height at 4 yrs of age, with one exception Gelbvieh are significantly lighter than females by other sire breeds. Results indicate that the reduced cow weight for Gelbvieh is associated with strong negative genetic trends for birth weight in the Gelbvieh breed, contrasted to slightly positive or null genetic trends for birth weight in other breeds. The lack of differences among breeds for cow weight and height, contrast sharply with comparisons made 30 years earlier when cows by Continental European sire breeds were on average 6 inches taller and about 30 lb heavier than those by British sire breeds.

The GPE program is used world-wide as the leading source of within and between breed genetic characterization data for designing beef cattle breeding programs. These data further enhance this database for the use by the beef cattle seedstock and commercial industries.

Characterization of tropically adapted beef breeds (2). Nine tropically adapted breeds have been evaluated in the Germplasm Evaluation Program at MARC in cooperation with ARS locations (Brooksville, FL; El Reno, OK) and with State Agricultural Experiment Stations (Texas, Louisiana) in the US. The breeds evaluated have included four *Bos indicus* (Brahman, Sahiwal, and Nellore with origins in India, and Boran from East Africa) breeds, three *Bos taurus* breeds (Tuli from Zimbabwe, Romosinuano from South America, and Bonsmara from South Africa), and two Brahman influenced breeds developed in the U.S (Beefmaster and Brangus). Results have shown that *Bos indicus* X *Bos taurus* F_1 cross cows (e.g., Brahman X Hereford, Brahman X Angus) are exceptionally productive and efficient as cows especially in subtropical regions of the U.S. Use of F_1 Brahman cross cows, Nellore, or Boran F_1 cross cows or rotational crossing of composite breeds such as Beefmaster, Brangus,

Bonsmara are especially well adapted to subtropical environments in the U.S. However, as proportion *Bos indicus* inheritance has increased, meat tenderness and marbling has been reduced; postweaning gain and feed efficiency has been reduced during winter months, and age at puberty has increased limiting conception rate in yearling heifer. In developing composite populations with an overall level of 50% tropical adaptation which has been more optimal in relatively humid subtropical regions of the U.S., it may be appropriate to substitute a portion (e.g., 25%) of non *Bos indicus* for *Bos indicus* from such breeds as the Tuli or Romosinuano to maintain tropical adaptation and improve meat tenderness, age at puberty and reproduction rate for young cows, provided they are crossed with other breeds that optimize size and growth rate.

Cow breeds differ in body weight maintained on fixed amounts of feed (3). Breed effects on weight maintenance at specific feeding rates and effect of breed and previous nutrition on the rate and efficiency of weight gain and loss of nonpregnant, nonlactating mature cows was determined in five breeds of beef cattle. Three breeds have been adapted for use in hot sub-tropical (Brahman, Boran, and Tuli) and two breeds are from temperate environments (Hereford and Angus). This study provided the information required to make breed adjustment in prediction equations for nutrient requirements. Previous studies using similar methodologies in other breeds have been incorporated into current feeding standards. Additionally, heat production across ages of beef females that vary in the proportion of Brahman breeding was quantified. Results revealed metabolic rate in cattle to be influenced by genotype and age and to be dynamic rather than static. Results of this study will help improve the prediction of energy requirements of growing beef females by accounting for breed differences in metabolic rate.

Expression of genetic effects in beef cattle can depend on the production environment (4).

Crossbreeding systems are used in commercial beef production to take advantage of hybrid vigor and complementarity among breeds of cattle. However, the expression of these genetic effects can depend on the production environment. The forage resources used in cow-calf production systems are dictated by the region's climatic conditions. These forages play an important role in the health and performance of the cow herd and the subsequent performance of the calves produced. 'Kentucky-31' tall fescue (*Festuca arundinacea* Schreb.) and common bermudagrass (*Cynodon dactylon* [L] Pers.) are major forages used for beef production throughout the southern United States. However, much of the tall fescue is infected with the endophyte *Neotyphodium coenophialum*. Cow and calves reared on endophyte infected fescue are on a lower plane of nutrition than cow and calves reared on bermudagrass pastures. As a result cow grazing infected fescue pastures produce less milk and calves reared by these cows weigh less at weaning. Cows and calves of Brahman breeding are more resistant to the toxins in endophyte infected fescue than calves of British breeding. Using Angus X Brahman or Brahman X Angus dams with a third breed as a terminal cross is an effective management tool to decrease the adverse effects of the endophyte infected fescue on animal performance. Although production systems that used infected fescue as the primary forage resource produced lighter calves at weaning, there were no carryover effects on stocker calf or feedlot performance.

Selecting beef cows for increased genetic merit for milk production increases forage intake requirement (5).

Milk production in beef cows has an important influence on the weaning weight of the calves and the efficiency and profitability of cow-calf enterprises. However, nutritional limitations imposed by the forage resources may prevent the expression of genetic potential for milk production. Selecting beef cows for increased genetic merit for milk production will increase the amount of forage dry matter consumed and will place additional pressure on forage resources. Using a mixture of tall-fescue and legumes will enhance forage quality and allow cows to express their genetic capacity for increased milk production. Alternatively, genetic potential of cows for milk production can be matched to existing forage bases by selection of cows based on levels of sire maternal weaning weight expected progeny differences consistent with existing production system forage resources.

Heritability estimates for carcass and palatability traits for Brahman cattle lead to tenderness EPDs (6). Over 500 Offspring from 27 Brahman sires were evaluated for various attributes including carcass quality and palatability traits. Heritability estimates for carcass traits (e.g., carcass weight, ribeye area, marbling score) were moderate to high suggesting that that selection would be effective for these traits. In contrast, heritability estimates for palatability traits (tenderness, juiciness, off flavor) were low suggesting that improvement by selection would be slow. In cooperation with Louisiana State University, carcass results from ARS Brahman research were combined with those from others to generate comparative breeding values (EPDs) for carcass traits and tenderness for the Brahman breed. These were published by the American Brahman Breeders Association in 2004 and 2005. Producers that use Brahman cattle now have the information to make informed selection decisions on bulls for improvement in carcass quality and beef tenderness.

Determination of variation among breeds of cattle in carcass composition and meat quality (7). Thirteen breeds of beef cattle were evaluated for meat palatability and carcass composition traits. These breeds included the seven most common in the U.S. and some from other countries that could improve on one or more traits relative to breeds currently available. This project has identified two tropically-adapted breeds that do not have tougher meat, and obtained an updated evaluation of the most commonly used breeds in the U.S. Collectively, this information has enabled producers to make better decisions on what germplasm to use to best utilize their resources for profitable beef production. The beef industry is rapidly moving towards use of carcass EPDs and data from these experiments will be the basis for developing adjustment factors for making across-breed EPD comparisons. This information has become even more important as market conditions change to increase emphasis on efficient production of lean and tender meat, and more companies are established to sell brand-labeled beef rather than commodity beef.

Molecular markers identified in beef breeds (8). Two DNA markers for the CAPN1 gene on chromosome 29 were characterized for their effects on beef tenderness in several different populations. This gene produces the protein mu-calpain involved in postmortem break down of protein and was previously located within a region associated with differences in beef tenderness in progeny of two bulls. Markers that had been developed within this gene were tested in several cattle populations to assess whether these markers might be useful in more general populations. Significant associations with tenderness were found in *Bos taurus* cattle but not *Bos indicus* cattle. A third marker was tested in *Bos indicus* and *Bos taurus* and was found to be associated with tenderness in both types of cattle. This information is useful to developers and users of commercial genotyping tests and several companies use and market this genetic test to cattle breeders. This was the first DNA test included in a national genetic evaluation of beef cattle.

Several chromosomal regions (QTL) associated with differences in growth, carcass, and reproductive traits have been identified. The include marbling, rib-eye area, reproductive hormone levels, and birth weight on chromosome 5 and testes weight and volume was chromosome 29. The set of QTL identified in this and predecessor projects are considered by researchers and companies to be a primary source of starting points for developing genetic markers for specific traits.

Some gene markers associated with carcass composition and beef quality in *Bos taurus* cattle were not associated with these traits in a genetically distant *Bos indicus* breed. The robustness of these associations in diverse populations of cattle was not known. Several markers in the DGAT1, Thyroglobulin, and CAPN1 genes previously found to be associated with carcass composition and meat quality traits in *Bos taurus* were tested in the Brahman breed. Some markers were not associated with these traits in Brahman cattle. If markers are to be used in *Bos indicus* populations, appropriate markers need to be developed and tested.

Markers for two genes associated with differences in beef tenderness can both be used to improve meat quality. It was not known if the effects of these two commercially available gene markers would add together or if the effect of one gene marker would be masked by the other. The markers were tested in *Bos indicus* and *Bos taurus* and crosses between these populations. Regardless of the population, the effects of the gene markers on tenderness were nearly independent and therefore both markers can be used to genetically improve tenderness. In populations where the alternative forms of both markers are present, cattle breeders can use both gene markers to attempt to improve beef tenderness.

Differences in growth rate and calf survival associated with a gene (myostatin) that results in highly muscled cattle were estimated in different genetic backgrounds (Belgian Blue, Hereford, Angus, Boran, Tuli, and Brahman). Results showed that the dam's genotype did not change the growth and carcass effect of the myostatin gene. If the myostatin allele was used for lean beef production, these results show that cows from a range of breeds could be used.

For animal paternity/identification markers, sequence tagged sites (STS) were developed from the expressed sequence tag (EST) effort and used to identify single nucleotide polymorphism (SNP) DNA markers. These markers were investigated for utility in determining parentage or in identification of animals for product traceback or other similar purposes, and the STS were characterized in depth to insure reliability of the genotyping systems in providing unambiguous results. The markers were among those used in proving that the December 2003 BSE index cow originated in Canada, having a multi-million dollar impact on the U.S. Beef Cattle industry. The markers may also be used in characterization of germplasm to increase the diversity of samples collected for any given breed.

Across-breed EPDs for beef breeds (9). Expected Progeny Differences (EPDs) have provided an effective tool for significant genetic change in direct and maternal components of growth to weaning and slaughter ages in most breeds of beef cattle, but EPDs can not be used to compare individuals of different breeds because they are computed separately by each breed association. A table of adjustment factors is estimated and updated each year using data from the Germplasm Evaluation Program at the U.S. Meat Animal Research Center. The Across Breed - EPDs (AB-EPDs), providing for comparison of animals from 16 different breeds on the same EPD scale, are most useful to commercial producers purchasing bulls of two or more breeds to use in systematic crossbreeding programs.

Characterization of sheep germplasm (10). Comparison of sheep breeds provides critical information to guide the appropriate use of breeds in crossbreeding systems. Superior productivity of the Romanov breed was documented, due primarily to greater conception rate, prolificacy, lamb survival, and longevity. During fall breeding, productivity of Romanov crossbred ewes was 24% greater than Finnsheep crossbred ewes and 63% greater than the average of crossbred ewes by Dorset, Texel, and Montadale. In spring breeding, Romanov crossbred ewes produced 55% more lamb weight than crossbred ewes by Finnsheep and 110% more weight than the average of crossbred ewes by Dorset, Texel, and Montadale. Due largely to these results, commercial sheep producers are benefiting by greater use of Romanov crossbred ewes in maternal roles of terminal crossbreeding systems.

In response to concern of commercial producers about the fertility and hardiness of traditional terminal sire breeds, a Composite population was created at MARC in 1980 by mating Columbia rams to Suffolk-Hampshire crossbred ewes. The high fertility of Composite rams in May through August breeding was documented in a large-scale commercial operation in California. Composite rams were group mated to over 1,100 commercial ewes in 2002 and 2003, resulting in conception rates of 83 and 92%, respectively. The high fertility of Composite rams during spring and summer breeding established that Composites are a viable option to traditional sire breeds. Consequently, ARS is providing greater producer access to Composite sheep as requested by the American Sheep Industry Association.

Determination of variation among breeds of sheep in carcass composition and meat quality (11).

Nine breeds (Dorset, Dorper, Finnsheep, Katahdin, Rambouillet, Romanov, Suffolk, Texel, and Composite) were evaluated for growth, carcass composition, and meat quality traits. Relative strengths and weaknesses of breeds across traits were documented revealing that no single breed excels for all relevant traits. This fact provides the basis for strategic use of breeds in structured crossbreeding systems. Efficiency of commercial lamb production is maximized in terminal crossbreeding systems by use of sire breeds to complement characteristics of crossbred ewes produced from general purpose and dam breeds.

Imprinted gene causing muscle hypertrophy in sheep identified (12). Identification of the mutation causing the callipyge syndrome in sheep, that causes increased muscle mass and decreased fat in affected animals. Genome sequencing of the portion of the sheep genome carrying the callipyge mutation was performed, the specific nucleotide sequence mutation was identified, and in collaboration with Duke University the novel gene in which the mutation occurred was discovered. Evidence that it represents a previously undescribed gene that appears to be conserved in humans, sheep, cattle, and mice was developed. Determining the function of this gene will represent a major breakthrough in the basic biology of the phenomenon known as imprinting, in which expression of a gene is dependent on the parental source. The muscle hypertrophy (enlargement) and reduction in carcass fat caused by inheritance of the callipyge mutation from the father (but NOT from the mother) is the only known case in mammals of a phenotype with this type of inheritance pattern. Characterization of the gene has had an impact on understanding of imprinting, and is highly likely to have ramifications in the study of muscle development, fat deposition, tissue homeostasis, and metabolism in humans as well as livestock. Furthermore, there are a number of human diseases associated with disruptions of imprinting at the equivalent portion of the human genome.

Characterization of swine germplasm (13). Characterization of breed, heterosis, and recombination effects are prerequisite to guide decisions for efficient use of genetic resources in crossbreeding systems. While considerable research has been devoted to breed and heterosis effects, estimates of recombination or epistatic effects are rare. These effects on reproduction, growth, and carcass traits were estimated from a large crossbreeding experiment involving the creation of two different four-breed composite populations of pigs. New epistatic combinations had few significant effects on reproductive traits, but effects tended to be favorable for white breeds that are widely used by the industry to produce F1 gilts. New epistatic combinations tended to produce neutral or favorable effects for growth and carcass traits.

MARC experiments have focused on the characterization of lines of pigs selected for 11 generations either at random, for ovulation rate or for uterine capacity. Selection was previously reported to increase ovulation by 3 ovulations and uterine capacity by 1 fetus per uterine horn, respectively, compared to the randomly selected control line. In this 5-year project, further characterization of fetal and placental development in these three lines has been completed. Fetal hematocrit at 105 days of gestation was greater in the uterine capacity line compared to the control line, supporting the concept that increased fetal erythropoiesis is associated with increased uterine capacity. However, neonatal hematocrit was significantly decreased in the uterine capacity line compared to control, suggesting that neonatal hematocrit would not be useful in selection for uterine capacity. Fetal brain, liver, heart and spleen weights have also been compared throughout gestation in the three lines. Results indicated (1) decreased liver and heart weights in the ovulation rate line, primarily due to extra crowding resulting from the greater ovulation rate, (2) no differences in organ weights in the uterine capacity line, and (3) increased "brain sparing" and decreased "heart sparing" effects with advancing gestation. The impact of these results is that although differences in organ development were not associated with selection for uterine capacity, significant mechanisms exist for shunting nutrients to various organs during gestation, and these might be exploited to improve uterine capacity. Finally, placental development has also been compared in the three lines. The pig placenta displays microscopic folding of the interface between

maternal and fetal epithelial components, and this arrangement along with other previously published results suggests that the depth of the folds influences the efficiency of maternal to fetal transport of nutrients. Morphometric analysis of placental histology indicated that (1) the depth of the folds begins to increase between day 65 and 85 of gestation, (2) the depth of the folds is greater in placenta associated with small, more compromised fetuses compared to their larger littermates, suggesting that this may be a compensation mechanism for lack of uterine space, (3) the stromal layer on the fetal side of the placenta becomes thinner with increasing gestation length, suggesting that the folds grow into this layer as gestation advances and (4) the stromal layer is thinner in small fetuses, suggesting that the width of this layer could become a limiting factor for continued increase in the depth of placental folding for small fetuses. Using proteomic analysis, we have identified several placental tissue proteins that increase between day 65 and 85 of gestation, some of these proteins could be involved in placental development. This research suggests a mechanism for fetal losses due to intrauterine crowding during late gestation. Results also suggest potential phenotypes (e.g., depth of folding, width of stroma) that could be exploited to improve placental efficiency, uterine capacity and litter size in swine.

Additional research determined that the secretion of follicle-stimulating hormone is greater during prepubertal development in gilts from lines of pigs that were selected for greater number of corpora lutea. During the follicular phase of the estrous cycle, a greater number of estrogen active follicles (ovulatory) existed on the fourth day after induction of luteolysis in gilts from the selected line relative to gilts from the control line. However, the ovulatory potential of neither line was achieved at estrus indicating that development of ovulatory follicles continues later into the follicular phase or that follicles ovulate at an earlier developmental stage than previously predicted. These findings, established in collaborative studies with the University of Nebraska, add to the understanding of regulation of ovarian follicular development in pigs and are helping to better define how litter size can be increased to enhance production efficiency.

Several MARC experiments have focused on the Meishan pig because this breed has been reported to possess increased uterine capacity. Previous ARS research indicated that a QTL for uterine capacity was located on swine chromosome 8. These findings were based on uterine capacity measurements in a population of Meishan-white crossbred gilts and the results indicated that the superior QTL allele(s) originated from the Meishan breed. Several candidate genes in the chromosome 8 region have been mapped. Comparison of endometrial expression of two candidate genes in the region between white crossbred and Meishan gilts and found significant differences in expression of both genes. The impact of this work has been an improvement in the ability to choose likely candidate genes in this region for further investigation.

Patterns of development of ovarian follicles and seminiferous tubules of testes were very similar in fetuses from commercial crossbred and Meishan sows. These findings were unexpected due to great difference in postnatal reproductive development of these two populations of pigs. Meishan males and females attain puberty at a much younger age than commercial crossbreds. Meishan sows have a greater ovulation rate whereas Meishan boars have lower sperm production relative to the crossbreds. These findings significantly impact the design of subsequent studies relating to determining the genetic basis of differences in gamete production in swine.

DNA markers studied in swine (14). A marker based on a polymorphism within the pig erythropoietin receptor (EPOR) gene was evaluated in both research and industry populations. The favorable allele has been associated with more than a one-pig difference in litter size. This effect would provide a substantial economic benefit to the swine industry but needs to be evaluated within current industry genetics. A cooperative agreement with Babcock Genetics revealed that the polymorphism was not segregating within this herd. Additional industry collaborators are being pursued. Other swine breeding companies are interested in evaluating this DNA test.

Alleles within a specific region of the X chromosome were shown to exert major influences on testis size during development in boars. In this region of the X chromosome, a single nucleotide polymorphism was found within the ligand-binding domain of the thyroid hormone-binding globulin (TBG) gene. The Meishan allele encodes for TBG that binds thyroxine with less affinity and is associated with smaller testes than the allele found in commercial breeds of swine. Thus, TBG may regulate testicular development thereby impacting sperm production of boars.

A panel of SNP markers was needed for the swine industry that could uniquely identify pigs and be used to determine parentage. An initial set of 40 SNP were selected that proved useful for these purposes in commercial pig populations. User feedback indicated additional markers may be necessary for certain circumstances. A second generation panel of markers has been developed containing 60 SNP markers.

Dairy cattle and goat genetic characterization toolbox enhanced (15). Specific cooperative agreements were initiated with four dairy records processing centers to obtain new data that will increase the value of the national dairy database as a research resource. Additional data categories include calf identification and pedigree, breeding and other reproductive events, and health incidents. Identification information submitted to the national dairy database early in an animal's life (as soon as an identification number is assigned rather than at first parturition) is more likely to be correct and available later when the calf matures to calve, because heifers often are sold without transfer of original pedigree information. Improved animal identification reduces the number of lactation records that are eliminated because of errors and increases data available for calculating genetic evaluations. Ready availability of edited data for reproductive and health traits will allow research to be conducted to determine feasibility of computing evaluations for those traits of economic importance and increasing genetic progress.

The national database for yield records of dairy cows and goats was modified to store test-day yields recorded since 1990 for dairy cows and goats. Incoming data are monitored to ensure that they are assigned to the correct herd and that testing characteristics match those reported for the herd. Various online queries have been developed to allow investigation and correction of data. Integration of lactation and test-day data minimizes data inconsistencies. Inclusion of goat data allows consistent processing of all data. Test-day data are used to calculate lactation yields for computation of national genetic evaluations. Those evaluations and research results depend on accuracy and completeness of the analyzed data.

Computer programs and electronic data-exchange procedures were developed and improved to provide an increasing number of routine reports that describe industry programs and practices as well as statistics from the Animal Improvement Programs Laboratory's genetic evaluation system. Current and past annual statistics on herd yield averages, State and national lactation averages by breed, participation in dairy testing programs, and activities by dairy records processing centers are available on the Laboratory web site. Genetic and phenotypic trends shown as averages by cow birth years are automatically produced and posted on the web site quarterly for milk, fat, and protein yields, somatic cell score, productive life, and daughter pregnancy rate as are statistics on bull and cow evaluations by breed and category. Those reports can be obtained through the Laboratory web site shortly after input data become available for use in educational and informational material by breed, artificial-insemination, and Extension cooperators; updates of reports are published routinely by many dairy magazines. Information on genetic trends is frequently used by university researchers to provide parameters for simulation.

Interest in crossbreeding has increased among U.S. dairy producers as a means to alleviate health and fertility problems by capitalizing on heterosis for those traits. Numbers and types of U.S. crossbred dairy cattle were determined for each breed cross from recent national data for herds with both purebred and crossbred cows that had been measured for yield (milk, fat, and protein), mastitis resistance (somatic cell score), longevity (productive life), and conformation (cow size). Breed differences, general and specific heterosis, and recombination losses were estimated for those traits and indicated that some crossbreds

are more profitable than purebred Holsteins. A breed composition code was developed to allow proportion of genes from each breed to be reported for crossbred animals, and methods to compute breed composition and coefficients of heterosis for crossbred animals are being tested. This economic analysis assists in identifying which crosses may be profitable. The Holstein breed's larger population size, greater range of genetic merit, and faster progress compared with other breeds could limit future use of crossbreeding, but current matings of Holstein cows to the best available Jersey and Brown Swiss bulls would eliminate inbreeding depression, reduce calving difficulty, and produce profitable, crossbred calves.

Problem Area IIB – Conserving and Preserving Genetic Resources

Problem Statement: Shifts in consumer demand and protection of livestock from disease challenges can be addressed if there is genetic diversity from which to choose. Currently, the U.S. has genetic resources that are at risk of being lost. Equally or more importantly the rate of inbreeding is increasing within breeds and strains of livestock and poultry. This increase in inbreeding lowers overall fitness and reduces genetic variation.

Committed Goals: 1. A secure repository of semen, embryos, oocytes, DNA, and tissue for potential use by industry and research. 2. Strategies for regenerating lost or endangered genetic resources. 3. Preserve genetically diverse reference populations of livestock and poultry are important.

Planned Approaches: 1. Survey genetic resources and assess the potential for genetic resources to be at risk of being lost. 2. Develop criteria for preserving genetic differences across populations. 3. Develop quality controls for germplasm entering the repository systems. 4. Maintain designated live animal populations, gametes, embryos and/or cells. 5. Develop strategies for re-establishing lost or endangered genetic resources.

Expected Outcomes: 1. Quantify the status of the nation's animal genetic resources. 2. Collect and preserve genetically diverse germplasm. 3. Methods to reintroduce cryopreserved germplasm.

Engaged ARS Locations: Beltsville, MD; Brooksville, FL; Clay Center, NE; El Reno, OK; Fort Collins, CO; Miles City, MT; East Lansing, MI.

Selected Accomplishments:

National Animal Germplasm Program off to a running start (1). Of primary importance in developing germplasm collections is the ability to determine which animals should be added to the collection. This problem was addressed by utilizing computed genetic relationships from pedigree data in a cluster analysis. The cluster analysis places more highly related animals in the same cluster. Using this approach provides the National Animal Germplasm Program (NAGP) with a road map detailing which animals should be collected for the repository and it is cost effective, especially when compared to molecular approaches. The cluster analysis used to evaluate within-breed diversity is also of utility to breed associations, as several associations have utilized their breed's cluster analysis to select animals for genomic studies.

Ownership patterns, based upon breeder registrations over time, were evaluated. Such analyses demonstrated that across major and minor breeds there are a relatively small proportion of breeders responsible for maintaining genetic diversity. For example, only 4% of the Navajo Churro sheep breeders have maintained flocks for more than seven years. Similar longevity patterns have been obtained for other breeds. Such information is critical in making in-situ conservation plans and targeting herd/flocks for the repository collection. In conjunction with species committees and the American Livestock Breeds

Conservancy surveys of swine, chicken, and turkey populations were performed. This information provides a baseline for future comparison of breed stability.

Development of the U.S. Country Report on animal genetic resources for the United Nation Food and Agriculture Organization (FAO) provides the first full assessment and synthesis of information concerning the state of livestock genetic resources in the U.S. The report provides information on breed level population trends and discusses those trends in the context of the livestock industry. As part of the FAO's State of the World's Animal Genetic Resources process we organized a Canadian and U.S. workshop that synthesized the similarities and differences between the two countries' animal genetic resources. The results of this workshop will be incorporated into the FAO report for the State of the World's Animal Genetic Resources.

NAGP and the American Dairy Science Association co-sponsored the 9th Discover Conference "Protecting and Managing Animal Genetic Resources for Future Generations: The Next Steps". This meeting allowed NAGP committee members, plus others interested in the topic, to meet and discuss the programs progress over the past five years, review the status of animal genetic diversity, and to develop a set of recommendations for the next five years.

To assist ARS in developing operational policies and collection activities six "species" committees were formed and consist of: Beef Cattle, Dairy Cattle, Swine, Poultry, Small Ruminants, and Aquatic Species. Committee membership includes persons from ARS, universities, industry, other government agencies, and non-governmental organizations. As a mechanism to coordinate the practices across species committees a Policy Coordinating Committee was developed and includes the chair persons from each species committee plus CSREES, the American Livestock Breeds Conservancy, and the ARS national program leader for animal production as members. The interaction of these groups has led to the development of collection strategies, identification of priority populations for collection, policies and procedures for acquiring proprietary populations, and the release of germplasm. The impact of these actions is a consistent and unbiased method of working with contributors and requestors. Additionally, the species committees have provided input to CSREES on genetic diversity and cryopreservation elements that can and should be addressed through the National Research Initiative Competitive Grants Program.

The acquisition of germplasm across species has exceeded project plan expectations by the metrics of populations, number of animals and quantity of germplasm and tissue (Table 1). As a result, germplasm collections for 17 populations have reached Core Collection levels (Core Collection is the quantity of germplasm necessary to regenerate a population by 150%) and are therefore secured in terms of our ability to reconstitute them. Therefore, the security of US genetic resources has been greatly enhanced.

In addition to the beef cattle germplasm stored in Ft. Collins (Table 1), ARS locations in Brooksville and Clay Center have, respectively, an additional 20,000 and 55,000 units of semen on a variety of *Bos Indicus* and *Bos Taurus* breeds.

ABS-Global, a company that sells dairy and beef cattle semen, contributed their historical semen collection to NAGP. This collection contained semen from all beef and dairy bulls the company had sold between 1960 and 1990. The collection consisted of approximately 6,000 bulls and 230,000 units of semen.

Frozen semen from nine breeds of sheep was delivered to the NAGP repository from the germplasm populations at the U.S. Meat Animal Research Center. Due to the recent sampling of rams from industry flocks, the collection conserves germplasm of these breeds as they presently exist. Over 21,000 straws of semen were processed from 154 rams representing the Dorset, Dorper, Finnsheep, Katahdin, Rambouillet, Romanov, Suffolk, Texel, and Composite breeds. The semen collection could be used to

measure future genetic changes of these breeds, to provide diversity if breeds become genetically limited, and to serve as a resource for genomic research.

Table 1. Status of Germplasm in the NAGP Repository

<i>Species groups</i>	<i>Species, breeds or lines (populations w/ tissue only)</i>	<i># Animals (animals w/ tissue only)</i>	<i>Units of (tissue)</i>	<i>Populations secured</i>	<i>Populations with >50% secured level</i>
Beef cattle	37	1,494	71,802	4	3
Dairy cattle	7	7,068	241,891	4	0
Chickens	80 lines	530 (72)	2,415 (783)	6	8
Aquaculture	25 species	240	9,126	1	0
Goats	11	178	4,593	0	0
Pigs	28	570	81,026	1	2
Sheep	21 (6)	338 (220)	31,252 (2,519)	0	0
Total	215	10,710	445,407	17	13

Table 2. Secured Populations

<i>Species groups</i>	<i>Populations secured</i>	<i>Specialized lines</i>
Beef cattle	Angus, Hereford, Salers, Simmental	Line 1 Hereford
Dairy cattle	Brown Swiss, Guernsey, Holstein, Jersey	U. Minn. Holstein Control Line
Chickens		Cornell – Line N ADOL Line 15B1 VT - High Antibody Response VT – Low Antibody Response VT – High 8 Wk Gain VT – Low 8 wk Gain
Aquaculture		Leetown - Rainbow Trout
Pigs	Yorkshire	Danbred

Germplasm acquisition and requests: The tissue collected to date came from over 300 individuals and/or companies. Three separate requests for dairy and beef cattle germplasm were received, reviewed and distributed. As a result, samples from 378 bulls from 12 breeds were released from the repository. All of these samples were used for DNA analysis. These first requests allowed NAGP to evaluate withdrawal protocols which are to be used across species. In addition, it demonstrates how the repository can be a source of tissue for scientists conducting DNA research.

Movement and Cryopreservation of Germplasm: Due to the lack of industry infrastructure and the varying use of cryopreservation, additional germplasm handling and cryopreservation methodologies are needed to facilitate collection, shipping and freezing of germplasm. Such techniques are critical for species where the utilization of cryopreserved semen is limited. For swine, a protocol was developed where

samples collected at studs or on farms can be packaged and shipped, at 15 degrees C, to NAGP. This procedure has been routinely used to acquire boar semen.

A number of protocols exist for cryopreserving boar semen, NAGP wanted to know which protocol would best serve our objectives. Therefore, an experiment was conducted to test three extenders and two breed types. It was determined that the BF5 extender yielded the best post-thaw motility and viability scores. As a result, this extender was chosen for routine cryopreservation of boar sperm. The protocol for the BF5 extender has been placed on the NAGP web site where it is available to any laboratory or company. Several entities have used the protocol in their work and as the protocol of choice in research proposals.

In order for the NAGP to obtain swine semen for cryopreservation at its germplasm storage center in Ft. Collins, CO, cooled boar semen must be transported for up to 24 hours from the site of collection to Ft. Collins. ARS researchers in the Biotechnology and Gamete Laboratory (BGL) conducted fertility trials to determine if the fertilizing ability of the spermatozoa was maintained after it had been cooled and stored for up to 24 hr prior to cryopreservation. The addition of a 24 hr cooling period before cryopreservation yielded an average pregnancy rate of 59% compared to 53% for the normal 3 hr cooling period with 7.9 live pigs born per sow. The results are important to swine producers, scientists, and the NAGP because they demonstrate that boar semen can be cryopreserved for the NAGP program with confidence that the spermatozoa will retain fertilizing capacity sufficient to repopulate in the event of a severe population loss or to introduce desired genetics into future swine populations.

BGL researchers also developed and tested an integrated group of technologies that would enable consistent cryopreservation and subsequent post-thaw viability of swine embryos. During FY2003, these technologies, collectively referred to as the 'USDA Swine Embryo Cryopreservation Technology', were granted US Patent protection (US Pat. No. 6,503,698). Patenting of the USDA Swine Cryopreservation Technology ensures its availability to the international swine industry and provides the basis for further improving and implementing embryo cryopreservation in swine breeding and genetic selection strategies.

There is a general lack of facilities to cryopreserve small ruminant germplasm. Therefore, an approach similar to shipping boar semen was developed. This was accomplished by evaluating ram semen at 5 degrees C for 24 and 48 hr prior to cryopreservation. It was determined that ram semen could be held for up to 48 hr without detrimental effect. A fertility trial was conducted with the University of Wyoming and showed there were no significant differences in fertility between semen that was inseminated: fresh, frozen immediately after collection, or frozen after a 24 hr holding period. These results provide confidence in collecting, shipping and freezing ram semen and have ramifications for the sheep industry to increase its utilization of cryopreserved .

A workshop on cryopreserving trout milt was held. This workshop established collaborative linkages between participants which have enabled germplasm to be collected and frozen for the repository. Specifically, it initiated collaboration between NAGP and the Colorado Division of Wildlife in developing a security backup for their breeding populations, including the Hoefer line of rainbow trout. The Hoefer line is believed to be resistant to whirling disease.

Problem Area IIC – Information Systems

Problem Statement: Genetic composition and performance must be integrated to fully understand the status and potential use of genetic populations. As germplasm collections increase, a critical need exists to develop databases capable of monitoring inventory (within and across locations), germplasm viability tests, and key genetic and phenotypic parameters associated with germplasm stored in the repository system.

Committed Goals: 1. Inventory of preserved germplasm and breed populations in the U. S. electronically linked to international information. 2. Evaluate genetic resources using information on breed performance related to production systems. 3. Develop software to assist in evaluating economic worth of genetic resources, determining genetic relationships among animals, and planning mating strategies.

Planned Approaches: 1. Develop decision support tools coupling genetic and economic methodology that enables industry and scientists to assess risk of losing genetic resources and to manage genetic diversity within a breed. 2. Develop databases to combine inventories of cryopreserved , scientific information on breeds and inventories of breed populations. 3. Develop information systems capable of evaluating changes in population demographics, differences in production systems and genetic by environmental interactions.

Expected Outcomes: 1. A database capable of determining the status of germplasm stored in repositories and linked to breed demographics. 2. A decision support tool for industry and genetic resource managers to use to evaluate the status of a population and to make decisions concerning maintenance or enhancement of genetic diversity. 3. An information system for tracking breed population trends over time and environments to interface with environmental and production variables.

Engaged ARS Locations: Fort Collins, CO; Clay Center, NE; Beltsville, MD.

Selected Accomplishments:

NAGP information system developed (1). Two principal aspects of information system (IS) development have been pursued, the development of a relational database and the development of a user-friendly interface that does not require the user to know Structured Query Language (SQL) but rather allows the user to explore the collection through any standard web browser. Development of a relational Oracle database was initiated by the Database Management Unit (DBMU) in Beltsville. DBMU was responsible for developing the tabular structure of the database using the NAGP staff for subject matter guidance.

The goals for this aspect of information system development were to build the database capabilities in the following areas: 1) the storage location of germplasm and the ability to add and subtract samples; 2) maintain records on the breeder and owner of the germplasm; 3) Summation of samples by animal, line, breed and species; 4) quality control measures of the germplasm (especially semen); 5) phenotypic, genotypic, and production system descriptors for animals; 6) processing of orders; 7) breed level information; and 8) the ability to handle multiple laboratories maintaining collections.

To date, 1-4 have been programmed and are functional and 5-6 are nearing completion. Germplasm and tissue that are acquired can be entered into and retrieved from the database.

A second aspect of IS development focuses on building a user-friendly interface using web-based technologies that allow the user to extract information from the database. The web pages can be accessed through any standard web browser that has access to the Internet. The first phase of this programming has been completed in Fort Collins, CO and users can view and explore the collection by species, breed, line, and individual.

RESEARCH COMPONENT III: GENETIC IMPROVEMENT

Genetic improvement of livestock and poultry populations is key to increasing production of high quality food efficiently and in an ethically responsible manner. The rate of improvement is compromised by lack of objective goals for improvement, inadequate understanding of quantitative and molecular genetic controls of component traits and interrelationships among traits, less than optimal methods for evaluating candidates for selection, and inefficient strategies to incorporate quantitative trait loci (QTL) in breeding programs, including the ability to move novel forms (alleles) of genes from one population to another.

Vision Statement: Genetically efficient and humane production of food from livestock and poultry.

Mission Statement: We seek to accelerate genetic improvement toward more efficient and profitable production of healthy, nutritious and palatable livestock and poultry products; and to improve health and well-being of livestock and poultry species.

Impact: Reduced cost of production and globally competitive animals and food products produced from livestock and poultry.

Linkages: USDA-ARS National Programs: 103 Animal Health; 105 Animal Well-Being and Stress Control Systems; 107 Human Nutrition; 205 Rangeland, Pastures, and Forages; and 207 Integrated Agricultural Systems

Other Agencies and Departments: Alcorn State University, Michigan State University, Montana State University, and the Universities of Arkansas, Idaho, Illinois, Nebraska, and Vermont.

Private Sector: National Association of Animal Breeders, Cattle Breed Associations, PIC, USA, Metamorphix, Inc., Anigenics, Beef Improvement Federation, and National Dairy Herd Improvement Association.

Problem Area IIIA –Develop Breeding Objectives

Problem Statement: Improving biological efficiency and profitability of livestock and poultry depends on changing several component traits in harmonious concert. These tradeoffs are not well established. Development of appropriate strategies is complicated by diversity of geographic, climatic, and economic environments in which production occurs and temporal variation within environments.

Committed Goal: Develop objective goals for genetic improvement for livestock and poultry species.

Planned Approaches: 1. Systems analysis of a farm-level production to gain new insight into selection goals for genetic improvement of biological efficiency and profitability of livestock and poultry. 2. Experimental validation of proposed objectives. 3. Incorporate new breeding objectives into genetic evaluation systems that can be used across breeds, strains, and lines.

Expected Outcomes: 1. Relative economic values for components of life-cycle efficiency linked with macro- and micro-systems for genetic evaluation. 2. Refined systems for local, national, and international genetic evaluations of livestock and poultry. 3. Genetic improvement technologies delivered to livestock and poultry producers.

Engaged ARS Locations: Beltsville, MD; Clay Center, NE; Dubois, ID; East Lansing, MI; El Reno, OK; Miles City, MT.

Selected Accomplishment:

Breeding objectives and selection indexes transferred to the beef seedstock industry (1). The Angus Sire Alliance, American Hereford Association, American International Charolais Association, North American Limousin Foundation, Agricultural Research Council - Animal Improvement Institute (Irene, South Africa) and Purdue University are using breeding objectives and selection indexes developed by ARS researchers. These indexes are used by beef cattle producers in making selection decisions that impact economic sustainability of individual farms and ranches and collectively affect profitability of the beef industry.

Enhancing dairy cattle genetic evaluation systems (2). The ARS Animal Improvement Programs Laboratory at Beltsville, MD has made significant contributions to dairy genetic evaluation procedures, including:

- 1) The International Bull Evaluation Service (Interbull, Uppsala, Sweden) combines genetic evaluations of dairy bulls across countries and provides rankings on each country's scale. The usefulness of foreign data in predicting later national evaluations was demonstrated for a number of countries. The application of estimated genetic correlations showed only marginal improvement in increasing accuracy of prediction of future national evaluations over an assumption of unity correlations between pairs of countries except for marked improvement for Australia and New Zealand. The accuracy of the Interbull procedure was found to be comparable, but not higher, than that of earlier methods of prediction equations developed for each country pair. A substantial portion of deviation of estimated genetic correlations from unity was shown to be explained by a modest degree of error in sire identification for cows. A procedure was developed for combining Interbull results to global or subglobal scales to facilitate use by nonparticipating countries and marketers. The repeated findings that combining data across countries improves prediction accuracy even though present procedures are not optimal has promoted confidence in use of the best information while encouraging improved methodology. Global rankings can be used by breeders in countries that do not participate in Interbull and by international marketers without referring to a particular national scale.
- 2) Prior to 2002, national genetic indexes for lifetime economic merit of dairy cattle did not include reproductive traits, which limited breeders' ability to select for overall production efficiency. The Animal Improvement Programs Laboratory revised net merit indexes to include calving ease and cow fertility traits and also updated economic values for other traits. The maternal grandsire effect for calving ease, which is called daughter calving ease, service sire effects for calving ease, and daughter pregnancy rate were added to the revised indexes for net merit, cheese merit, and fluid merit. Selection of animals to be parents of the next generation of U.S. dairy cattle based on the new lifetime merit indexes that include economically important reproductive traits will result in a more efficient and profitable dairy population.

Problem Area IIIB – Accelerate Selection Response

Problem Statement: Cumulative and sustainable change in populations is achieved through selection. Rate of response to selection is compromised by sub-optimal systems of genetic evaluation, incomplete genetic characterization, interactions of genotype with environment, and genetic antagonisms among traits. Knowledge of genes affecting production traits and how these genes interact with the rest of the

genome and the production environment, will enable breeders to make selection decisions that expedite genetic progress and target specific traits limiting efficiency.

Committed Goals: 1. Enhanced systems for genetic evaluation of livestock and poultry. 2. Improved understanding of genetic architecture controlling expression of traits influencing biological efficiency and profitability. 3. Increase biological efficiency and profit for livestock and poultry producers through more optimal selection decisions.

Planned Approaches: 1. Upgrade systems of genetic evaluation, incorporate QTL information, and evaluate selection response to marker assisted selection. 2. Develop statistical methods for QTL detection and characterization in complex livestock pedigrees. 3. Partition phenotypic variance into causal components, identify QTL for traits in breeding objectives (fertility, fitness, growth, composition, nutrient density, etc.), and establish quantitative and molecular genetic relationships among traits. 4. Identify impacts of diverse production environments on estimated genetic parameters and breeding objectives. 5. Assess direct and correlated responses to selection.

Expected Outcomes: 1. Identification of significant genetic antagonisms between components of life-cycle production efficiency in livestock and poultry. 2. Deliver technologies for accelerating genetic improvement in efficiency and profitability to livestock and poultry producers.

Engaged ARS Locations: Beltsville, MD; Clay Center, NE; Dubois, ID; East Lansing, MI; M. City, MT.

Selected Accomplishments:

Variation in feed utilization will allow for genetic selection (1). Feed intake and animal performance data were collected utilizing individually fed steers of diverse genetic and nutritional backgrounds. Results demonstrated that a large amount of variation exists among individuals in efficiency of energy use for body weight or body energy gain, both within and between breeds of cattle. Results indicate that genetic selection for efficiency of nutrient use can potentially be used to increase nutrients retained by the animals and decrease nutrient loss from animals. These findings have been used to identify phenotypic measures that can be used in a long-term study to find QTL associated with feed efficiency. This study has three primary objectives: 1) to develop approaches to distinguish growing-finishing castrate males having efficient and inefficient phenotypes; 2) to identify and measure molecular and quantitative variation affecting biological efficiency and its components of efficiency, production traits, and product value; and 3) to identify QTL associated with important components of production efficiency. Information from this study will be used to identify individual animals that have a high likelihood to be efficient in feed utilization, develop tools to identify the most appropriate feeding strategies for an individual animal, and contribute to selection indexes to evaluate potential animals for breeding.

Use of plasma urea nitrogen (PUN) as an index of efficiency of dietary amino acid utilization and adequacy of dietary amino acid nutriture (2). Analysis of PUN and growth data from a large sample of animals revealed influences of age, sire, sire line, and sex on PUN and a heritability estimate of 0.35. This PUN work was awarded the 2003 National Pork Board Research Innovation Award. Other PUN results indicate selection against swine with high PUN may result in swine with lower PUN, and, consequently, reduced excretion of urea nitrogen and a minimal or positive impact on growth and yield of pork measures.

Survival of calves and weights of cows are important components of cattle efficiency (3). A selection experiment for increased calving ease while restricting change in yearling weights showed that calves born to two-year-old heifers had improved survival from birth to weaning and that five-year-old cows weighed 4 percent less. Results demonstrate to cow-calf producers that selection can

simultaneously reduce calving difficulty, improve calf survival, and reduce cow weights while maintaining growth to one year of age.

Selection successful for increased reproductive rate in cattle (4). Genetic selection of cattle (MARC cattle twinning population) for increased ovulation rate, and consequently increased female gamete production, increased the number of gametes 0.026 oocytes/estrous cycle/year of selection. Ovulation rate was measured in all puberal heifers for six consecutive estrous cycles. These results demonstrate the effective application of using repeated measurements of a phenotypic trait (e.g., ovulation and twinning rate) and multiple-trait, repeated-records data analysis and/or marker-assisted genetic selection to improve reproductive traits with a low heritability estimate. The marker-assisted selection protocol utilized information for quantitative trait loci (QTL) linked to ovulation rate and twinning rate found on chromosomes 5 and 7.

ARS contributes to transfer of DNA marker technology to the beef industry (5). The National Cattlemen's Beef Association conducted a QTL validation study as part of the National Carcass Merit Project (CMP), a large, industry-wide progeny testing project for tenderness and other meat quality traits. An analysis of the CMP data for QTL affecting meat quality traits was conducted. Results were reported to the 14 respective breed associations. The ARS contribution to the CMP effort was through independent validation analysis of the results in this project.

The effects and utility of published DNA tests associated with performance tests in cattle are often difficult to assess for U.S. beef cattle producers because some genotype classes are at low frequency in test populations. In order to rectify this deficiency, selection for rarer genotypes in the base cow herd(s) at MARC has been carried out, which currently involves testing several hundreds of proposed selection bulls and cows with seven published DNA markers (SNP) in six cattle genes (diglycerolacetyl transferase (DGAT1), calpastatin (CAST), growth hormone receptor (GHR), CSN1S1, u-calpain (CAPN1), thyroglobulin (TG)). This is a slow process due to the long generation interval, but some progress has been made and it is expected in the next 5-year cycle to be able that more accurate assessment of the utility of these markers for accelerating selection response will result.

ARS researchers advised and participated in the National Beef Cattle Evaluation Consortium's (NBCEC) efforts to develop strategies for the incorporation of DNA test results into national genetic evaluations. As a part of this effort, they reviewed and suggested analyses and contributed to the development of policy on implementing independent validation and reporting the results. The independent validation process provides cattle breeders, breed associations, and extension specialists with unbiased information to aid them in deciding whether to use or recommend specific DNA tests. The major DNA testing companies participate in the process because their customers have come to expect it. Contributions were made to the first such evaluation (American Simmental Association's evaluation of tenderness considering DNA test results for mu-calpain). The consortium is comprised of the four universities (Cornell, Colorado State, University of Georgia, and Iowa State) that currently conduct national cattle evaluation for the beef industry and an advisory council including leading beef producers, breed association staff, and extension specialists.

Bovine genome sequence draft assembly used to confirm QTL associations in targeted region (6). ARS researchers at the U.S. Meat Animal Research Center were one of the first groups to utilize available bovine genomic sequence to target a rather large QTL region for marker development to confirm associations with previously identified QTL. Because of the fragmentary nature of the first available sequence (~3-fold sequence coverage) only a low level coverage of the QTL region was obtained; however, demonstration of genotype/phenotype associations was achieved. This may impact future studies where marker density is a concern due to marker availability or genotyping cost.

Swine QTL resolution enhanced (7). Genetic markers that are accurate predictors of an animal's breeding value for important traits can be used in selection programs to increase the accuracy of selection. To develop markers for this use, genome scans have been conducted to identify QTL for reproduction and pork quality traits with numerous genomic regions detected for most phenotypes. Verification of reproductive QTL was conducted in subsequent generations of the MARC Meishan cross population as well as in lines of domestic germplasm selected for increased ovulation rate or uterine capacity. Verification of pork quality QTL is being conducted in a research population that is representative of commercial pigs.

Improving the resolution of QTL position permits selection and testing of positional candidate genes and develops markers that are more robust for selection decisions. These fine-mapping experiments have been conducted for reproduction QTL on swine chromosomes 3, 8, 10 and X as well as for pork quality QTL on chromosomes 2 and 17. One DNA variant that may be the causative SNP has been detected. A variant of the thyroxine binding globulin was identified that is associated with a 30-40 % reduction in testes size, reduced sperm production and various other biological factors studied in this population. The undesirable allele is the "ancestral" allele and clearly indicates selection for leaner pig and boars with larger testes has removed the undesirable allele from most commercial pig populations.

New data analytical tools reveal potential for selection for reduced lamb mortality in sheep production (8). Lamb mortality is a major constraint in sheep production systems around the world. Methods to genetically improve lamb survival were investigated by analyzing data collected on a terminal sire composite flock at MARC. Nontraditional methods of analyzing data detected greater genetic control of lamb survival than the common method of analysis. Use of these methods by the sheep industry will increase lamb survival, leading to improved productivity, profitability, and sheep welfare.

Enhancing dairy cattle genetic evaluation systems (9). The ARS Animal Improvement Programs Laboratory at Beltsville, MD has made significant contributions to dairy genetic evaluation procedures, including:

- 1) To support development and implementation of a test-day model for the U.S. dairy industry, a new method for detecting and adjusting for abnormally high or low (outlier) yield on test day was developed and implemented. Methods to account for a random herd effect on lactation curves were investigated to adjust for heterogeneous variances in a test-day model. National bull evaluations were compared with regional evaluations based on a test-day model to assess ability of evaluations to predict milk yield of future daughters; despite test-day methodology, regional rankings were found to be less useful than national animal-model evaluations in predicting daughter performance. Increased reliance on regional test-day evaluations would reduce current rate of genetic gain. Detection and correction of information from abnormal test days improved data quality for genetic evaluations; lactation yields across parities are more similar, and data from cows that are sick on test day are treated more consistently. The method to adjust for heterogeneity of components of variation provides dairy producers with more accurate animal rankings for making breeding decisions.
- 2) Including information from multiple parities in genetic evaluations has increased over the last two decades and is now standard practice for many countries. However, if genetic differences in maturity rate of daughters have a large impact on ranking of bulls, a modification of the evaluation model could be needed to treat yield from different parities as different traits. Differences in maturity rate of bulls' daughters were examined to determine how they impact change in bull evaluations across time. Bull evaluations based on specific parities were calculated, and bulls were found to differ in maturity rate of daughters as reflected by daughter milk yield. Evaluations based on designated parities were more stable across time than were official evaluations. If daughter maturity rate is confirmed to be genetic, modification of the

evaluation model to account for that trait should improve stability and accuracy of yield evaluations. Such a modification should reduce variation in evaluations across time, thereby raising breeder confidence that released evaluations were reliable estimates of the true genetic merit of bulls.

- 3) Many dairy cattle producers have expressed concerns about the difficulty of achieving desired levels of reproductive performance in today's milking herds. In response, ARS developed and implemented the first U.S. national genetic evaluations for cow fertility. After examination of several reproductive traits to determine if genetic selection for daughter fertility was possible, daughter pregnancy rate (the percentage of nonpregnant cows that become pregnant during each 21-day period) was chosen as the trait to be evaluated so that herd managers could measure how quickly their cows become pregnant again after having a calf. Pregnancy diagnosis was added as an additional source of data for predicting days open, and the evaluation system also was enhanced to use days open information earlier during lactation. Inclusion of predicted days open allows for earlier genetic evaluation of cattle, especially young bulls. The new evaluations for daughter pregnancy rate provide breeders with a tool for maintaining and improving cow fertility.

Problem Area IIIC – Improve Health and Well-Being

Problem Statement: Disease compromises both health and productivity of livestock and poultry. Populations vary in resistance to specific pathogens and genetic variation may exist within populations. Inbreeding is an unavoidable consequence of directional selection. Due to natural genetic load within populations, inbreeding usually results in reduced reproduction, vigor, fitness, and physiological efficiency.

Committed Goals: 1. Reduce disease and use of pharmaceuticals in livestock and poultry production. 2. Enhance fitness and well-being of livestock and poultry.

Planned Approaches: 1. Identify genes or closely linked markers related to production and disease traits. 2. Identify genes with joint effects on disease and production. 3. Assess effects of inbreeding concurrent with accelerated genetic improvement. 4. Develop strategies to maintain fitness and allelic diversity in populations of livestock and poultry under directional selection.

Expected Outcomes: 1. Increased resistance of livestock and poultry to disease and infection. 2. Maintain allelic diversity and improve fitness in livestock and poultry populations. 3. Delivery of genetic technologies to improve animal fitness, health and well-being in a variety of systems being used by livestock and poultry producers.

Engaged ARS Locations: Beltsville, MD; Clay Center, NE; Dubois, ID; East Lansing, MI; Miles City, MT.

Selected Accomplishments:

Identification of three Marek's disease resistance genes and numerous QTL (1). With Marek's disease costing the poultry industry over an estimated \$160+ million a year, developing methods that augment current vaccinal methods is a high priority. Using an integrated strategy of QTL mapping, gene transcription profiling, and virus-host protein interaction screens three disease resistance genes have been identified, along with numerous insights on the immune response that promotes genetic resistance. Genetic markers for these genes and markers for QTL are being evaluated in commercial layers to see if they enhance disease resistance.

Molecular assays developed to determine genetic resistance to avian leukosis virus (ALV) in chickens (2). There are six subgroups (A-E, J) of ALV. Each ALV subgroups imposes a varied degree of threat to the poultry industry. All ALV subgroups, except subgroup J, utilize a unique chicken cellular receptor for viral entry. ARS scientists developed molecular assays that determine the genetic makeup of individual chickens with respect to ALV cellular receptor status. Chickens lacking the genetic ability to produce the receptors are genetically resistant to the corresponding subgroups of viruses. This crucial technique empowers the genetic determination of genetic resistance of chickens at early stage of life (day one after hatch), and allows direct genetic selection in establishing chicken lines genetically resistant to specific ALV subgroups.

A genome-wide analysis to detect QTL completed for six Holstein grandsire families from a historic population identifies fitness QTL and leads to additional genome scans in cattle and sheep (3). Previous ARS research detected the genomic locations of production and health-related QTL in popular, historic Holstein grandsire families. In this work, large Holstein bull families were studied at over 200 DNA markers across the entire genome to identify DNA regions associated with economically important traits. The Cooperative Dairy DNA Repository (CDDR) was initiated during this effort to detect QTL for new phenotypic traits of interest (i.e. daughter pregnancy rate, milking speed, calving ease, etc.). Recently, a collaborative effort between ARS and the University of Illinois combined genotypic data from previous independent analyses (over 350 markers) to provide the most thorough analysis of QTL segregating in the U.S. Holstein population. More importantly, this was the first report of novel QTL for pregnancy rate in U.S. Holsteins. Some of the genomic positions of these QTL overlap with those reported by researchers working on ovulation rate QTL in the MARC cattle twinning population. Detection of pregnancy rate QTL is the first step in developing marker-assisted selection programs to improve genetic gain for fertility in cattle, which continues to be a major concern of the dairy industry. Additionally new SCS QTL, a trait correlated with mastitis incidence, were also confirmed for four different chromosomal locations.

Other genome scans completed or underway in new populations include: 1) completion of a genome scan in a novel Holstein family from the CDDR segregating for conformation and type QTL; 2) completion of genome scan for parasite indicator traits in the ARS BeltsvilleWye Angus herd (11 QTL on 8 chromosomes); and 3) nearly completed a genome scan of a sheep population developed using a back cross design derived from Dorper and Red Masai sheep that were phenotyped for worm fecundity and feeding (e.g. fecal egg count in feces and packed blood cell volume). In this latter project (a collaboration with the International Livestock Research Institute) ARS has genotyped six chromosomes from the sheep population that would be synonymous with the QTL initially detected in cattle. Preliminary analysis revealed that a sheep QTL for fecal egg count was located in a position synonymous with a similar QTL for FEC found on Chr 6 in the ARS Beltsville Angus herd.

The Cooperative Dairy DNA Repository (CDDR) continues to grow and now contains DNA samples from over 20,000 bulls (4). ARS scientists and scientists from academia and the private sector use this collection for their fine-mapping studies and validation of QTN. For example, ARS scientists have evaluated the production and economic values of selecting dairy animals based on the “high fat” and “low fat” alleles of the bovine *DGAT1* gene, which accounts for ~30% of the standard deviation in fat percentage in milk in Holsteins. This genetic information was used to complete an analysis that found profitability for milk producers could be increased by over \$30 per animal lactation by selecting for animals with the “low fat” allele. Originally, BFGI scientists thought QTN similar to that for *DGAT1* would continue to be analyzed using the CDDR resources, which should help establish a framework for developing gene-based MAS programs for U.S. producers across multiple traits. An additional gene encoding a transcription factor involved in T-cell activation (*FEZL*) has also been evaluated in this manner. The results strongly suggest this mutation was only a marker linked to the actual QTL for somatic cell score found on Chr 22. Ultimately, the impact of this type of research for the dairy industry hinges upon the development of reliable and robust marker-assisted selection schemes.

Today, outcomes from the bovine genome sequencing project are altering the relevance of these efforts and efforts in further mapping for production traits. The biological importance of finding and understanding causative genetic variation still remain but hold less importance relative to impact on the dairy industry. The marker resources generated from the bovine genome sequencing project make genome selection based on dense SNP markers the most effective, yet untested, route to developing reliable and economically feasible MAS programs that can be applied a commercial basis. As such, research on genome selection will be the major focus of future research efforts, and these efforts are fully endorsed by the dairy industry.

Osteopontin (OPN) contains a potential QTN for milk protein percentage in its promoter region (5). *OPN* expression in bovine mammary gland has been evaluated during different phases of mammary gland development and function. Experiments were initiated to express *OPN* promoter variants in cultured mammary epithelium to assess differences in transcriptional response that may provide mechanistic support to the putative QTN in *OPN*. Such validation should enhance the reliability of this marker for genetic selection of protein production.

Reproductive fitness QTL studied in beef cattle (6). A SNP discovery was performed in a selected group of animals (MARC twinning population) segregating or not segregating for putative QTL for twinning and ovulation rate on chromosome 5. The SNP data were merged with the “twinner” marker database and analyzed using GenoProb software (a program developed at MARC). Twenty-four SNPs were identified in 11 genes within this putative QTL region on bovine Chromosome 5. These results indicate great potential for accelerating fine mapping of QTL linked to production traits in beef cattle.

Genomic analysis of F₁ bulls produced from one Hereford x Brahman sire identified QTL for testes size on chromosome 29 and for follicle-stimulating hormone on chromosome 5. These are the first regions of the bovine genome to be associated with male reproductive traits in cattle and will improve procedures for selection of herd sires to meet specific production goals of producers.

Slick hair gene identified in cattle that enhances adaptability to heat stress (7). Evidence was found that supports the existence of a major gene (designated as the *slick hair* gene), dominant in mode of inheritance, that is responsible for producing a very short, sleek hair coat. Research evidence developed by ARS scientists in Brooksville, FL in cooperation with the University of Florida using Senepol crossbred cows and Carora cows (a milking cattle breed from Venezuela) supports the existence of a single, major, dominant gene that is segregating in their calves. Furthermore, rectal temperatures were lower in slick-haired Senepol crossbred calves and slick-haired Carora crossbred cows when compared to their normal-haired contemporaries. The incorporation of the slick gene into temperate breeds could have a major effect on productivity of those cattle in warm climates, particularly fertility of dairy cows through increased embryo survival and greater milk production during periods of heat stress. Incorporation of slick hair into temperate breeds of beef cattle should allow them to be raised successfully under conditions with greater heat stress than was previously possible.

Study of the callipyge condition leads to identification of important mutation (8). The callipyge mutation in sheep is important because very large effects on muscle and fat development, carcass leanness, and meat quality occur only when lambs receive the mutation from their sire and the normal form of the gene from their dam. Discovery of the callipyge mutation by ARS scientists revealed a previously unknown gene that obviously affects muscle and fat development. The mutation alters expression of multiple genes within an imprinted region, a genetic phenomenon of considerable interest in human disease research. Discovery of regional control on gene expression has extensive implications for all livestock species.

Identification of QTL affecting meat quality (9). Ongoing collaboration among scientists in the Meats Research Unit, Genetics and Breeding Research Unit, and the Molecular Genetics and Research Unit at

the U.S. Meat Animal Research Center has identified QTL affecting beef quality. A series of studies have confirmed an association between SNP in the μ -calpain gene and beef tenderness. This marker information has been commercialized and is being used by the U.S. beef industry. Additional QTL have been identified affecting pork meat quality. Validation and further refinement of these QTL's will pave the way to genetic selection for improved eating quality of pork. Finally, this group has identified QTL affecting both lamb loin chop tenderness and biochemical traits relating to meat tenderness. Identification of the genes responsible for these associations will allow for genetic selection for tenderness in lamb and should aid the search for genes responsible for variation in beef tenderness.

Problem Area IIID – Produce and Evaluate Transgenic Livestock and Poultry

Problem Statement: During the past 15 years procedures have been developed to introduce transgenes into genomes of livestock and poultry. This technology provides the opportunity to introduce new traits or modify existing traits that cannot be accomplished by genetic selection.

Committed Goal: Introduce transgenes into livestock and poultry that have potential to impact immunity or resistance to disease, increase product quality or safety, and increase efficiency of animal production.

Planned Approaches: 1. Produce and evaluate transgenic animals and birds that contain modified native, foreign, or synthetic genes. 2. Determine the impact of the introduced phenotype on efficacy, safety, and animal well-being.

Expected Outcomes: Provide producers with livestock and poultry with improved product quality or safety, increased resistance to disease, and enhanced production efficiency.

Engaged ARS Locations: Beltsville, MD; Clay Center, NE.

Selected Accomplishments:

Production of genetically-enhanced dairy cattle resistant to mastitis (1). Mastitis costs the dairy industry \$2B annually with the most deadly form of the disease caused by *S. aureus* infections. ARS scientists at Beltsville, Maryland have successfully produced, by nuclear transfer, genetically-enhanced cattle carrying the gene encoding lysostaphin, a potent killer of mastitis-causing bacteria. Working collaboratively with scientists at the University of Vermont, the research team has confirmed that lysostaphin is produced in the milk of the transgenic females and does confer resistance to *S. aureus* infection. The response to *S. aureus* challenge of three transgenic founder females and one G1 female offspring of a male founder during first and second lactations is the same. The level of protection is correlated with the level of transgene expression. Scientists now believe that a concentration as low as 3 micrograms/ml of lysostaphin is adequate to protect a cow from *S. aureus* caused mastitis. This is the first demonstration of using genetic engineering to protect a production animal against an economically important disease and suggests the approach could be used to protect livestock against other important diseases.

More work needed before swine endometrial cells can be cultured to test gene constructs (2). MARC researchers proposed to develop a uterine endometrial epithelial culture system that was capable of secreting uteroferrin, and then use this culture system to test expression constructs based on the uteroferrin gene. A construct designed to increase the secretion of secreted folate binding protein by placing it under the control of the uteroferrin promoter was successfully created. The next stage of the project required testing of the construct in the endometrial epithelial cell culture system. The culture method proposed was based on a report in the literature of a method of isolation and culture of these cells that would maintain uteroferrin secretion. The reported procedure was utilized, but researchers

found that as soon as the cells were isolated, they began to cease both uteroferrin gene transcription and uteroferrin protein production, a result that is consistent with other attempts to isolate and culture swine endometrial epithelial cells. No other suitable alternative isolation and culture method has been reported that will result in maintained uteroferrin secretion. The impact of these results are that transgenic animals based on uterine secreted genes, as proposed in the project, are not feasible until a suitable cell culture system is developed for initial testing of constructs.

Transgenic mice evaluated as model for swine for increased lean, decreased fat accretion (3).

Dual energy X-ray absorptiometry (DEXA) was used to evaluate transgenic mice expressing the myostatin pro domain gene that is thought to interfere with myostatin function and thereby promote muscle growth. DEXA analysis of the animals indicated that in males expressing the myostatin pro domain an increase in lean mass was initiated between 27 and 34 days of age. The increase in lean mass was accompanied by a small decrease in fat mass and small increases in bone content and bone density.

RESEARCH COMPONENT IV: GENOMIC TOOLS

Knowledge of the genome and its interactions with environmental factors are required to fully understand the biological basis of all animal science disciplines. Acquiring new knowledge pertaining to reproductive efficiency, genetic improvement, nutrient intake and use, and growth and development will be slow and inefficient without appropriate genomic tools. Both public and private laboratories will develop these tools. Public involvement in the construction of these resources is critical to ensure development of economically feasible management tools for livestock producers and to provide all researchers access to these tools and technologies.

Vision Statement: Provide a comprehensive set of genomic tools for food animal production research.

Mission Statement: We seek to develop genomic and proteomic tools and information to facilitate research in livestock and poultry species.

Impact: Rapid progress in elucidating the structure and function of genes.

Linkages: USDA-ARS National Programs: 103 Animal Health; 104 Veterinary, Medical, and Urban Entomology; 105 Animal Well-Being and Stress Control Systems; 106 Aquaculture; 108 Food Safety.

Other Agencies and Departments: USDA-CSREES, NAAB, EMBRAPA, INRA, New Zealand Livestock Improvement, BBSRC, The Roslin Institute, Shirakawa Institute of Animal Genetics, Korean Livestock Institute, Cold Spring Harbor Laboratory, Genome Canada, State of Texas, Beef Industry Council, National Pork Board, National Cattlemen's Beef Association, The Institute for Genomic Research, NIH, NIDDK, Cornell University, Duke University, Hebrew University of Jerusalem, Montana State University, North Carolina A&T, and the Universities of California, Delaware, Illinois, Minnesota, and Nevada-Reno.

Private Sector: Sequenom, Inc., Merial, Ltd., Metamorphix, Inc., and Chapman Bonsmaras.

Problem Area IVA – Comprehensive Maps

Problem Statement: Genetic and physical maps for a few livestock species exist today, but more comprehensive genomic maps for livestock and poultry still need to be developed. Comparative maps that tie to the mouse and human maps are also needed. These maps need to possess types of markers that facilitate use of high-throughput genotyping platforms. High-resolution maps would greatly expedite sequencing of the entire genome of food animal species, yielding the ultimate physical maps. Sequenced genomes have revolutionized investigations into comparative biology, genomics, genetic marker development, and gene identification experiments.

Committed Goals: 1. Develop dense genetic linkage maps and comprehensive physical maps for livestock and poultry. 2. Sequence the complete genomes of livestock and poultry species.

Planned Approaches: 1. Develop and map microsatellite and single nucleotide polymorphisms in well characterized reference populations. 2. Develop bacterial artificial chromosome (physical) maps for agricultural species and link these maps to genetic and physical maps of livestock and other species. 3. Sequence approximately 3 billion bases of genomic DNA per livestock species and 1 billion bases in chickens. 4. Compare sequence information among species, including mouse and human.

Expected Outcomes: 1. Linkage maps and physical maps with sufficient marker densities that will be useful in a wide range of populations. 2. Relatively complete physical (BAC) maps for food animal genomes. 3. Complete sequence coverage for livestock and poultry genomes.

Engaged ARS Locations: Beltsville, MD; Clay Center, NE; East Lansing, MI.

Selected Accomplishments:

Draft annotated assembly of the chicken genome sequence made available to public domain (1).

The Washington University Genome Sequencing Center, St. Louis, MO, sequenced the genome of a Red Jungle Fowl (RJF) chicken to 6.4-fold coverage and deposited the information at NCBI in March 2004. To assemble the draft genome sequence, sequences were aligned in part to the genetic map developed by ARS. This is the first avian species, and the first of the domesticated agricultural species, to be completely sequenced. The sequence has been further finished with funding from USDA-ARS, resulting in a second release of the draft sequence in December 2005 to 7.3-fold coverage. This powerful tool provides the complete “parts list” for a chicken and is the basis for understanding the molecular basis for all inherited traits, greatly facilitating future poultry breeding for superior birds.

Toward a chicken SNP haplotype map to enable genome-wide marker-assisted selection and individual bird traceability (2). A fundamental question in biology is how genetic variation leads to phenotypic (observable) variation. With the advent of the draft chicken genome sequence, it is now possible to identify sequence variation between birds. As part of an international consortium, >2.8 million putative single nucleotide polymorphisms (SNPs) were revealed from light sequencing of three lines (broiler, layer, and Chinese silkie) as compared to the base RJF sequence. ARS researchers are now leading a large project to characterize 3,072 of these SNPs by genotyping over 2,500 birds from commercial and experimental lines. The results are addressing: (1) the validation and allele frequency of the SNPs, (2) the generation of a high-density SNP genetic map and its integration with the genome sequence, (3) feasibility of genome-wide marker-assisted selection in commercial chicken lines, and (4) determination of what subset of SNPs would allow traceability of poultry meat or live offspring to its pure-line parent. This project is leveraged with funds from the USDA-National Research Initiative Competitive Grants Program.

Bovine bacterial artificial (BAC) chromosome map developed (3). A bovine physical map comprised of ~300,000 fingerprinted BAC clones and ~75,000 BAC-end sequences was developed based upon DNA from an inbred Hereford bull from the Line 1 germplasm population at the ARS Fort Keogh Livestock and Range Research Lab. This map reduces the time required to identify markers for a gene, which was previously about one year and is now matter of a few weeks. The map, which provides approximately ~17-fold coverage of the bovine genome, is being used for fine mapping, assembling the sequence, selecting clones for sequencing, annotating the sequence, functional genomics and cloning. The BAC map was used to select the minimum tiling path of clones for genomic sequencing in the effort at Baylor College of Medicine (see below). Many scientists are using the BAC map to develop markers for QTL, which may lead to marker-assisted selection, improved management, or pharmaceutical targets. This project was a collaborative effort undertaken by the International Bovine BAC Map Consortium, led by ARS scientists and funding, and with participating members from INRA, Embrapa, BBSRC, Genome Canada, New Zealand Livestock Industry Council, CSIRO, Texas A&M, and the University of Illinois.

Integrating bovine linkage and physical maps into a more valuable one-stop resource (4). The number of microsatellite DNA markers on the MARC cattle linkage map was increased by 2,300 to a total of 3,800 individual markers in a collaborative effort between ARS scientists, University of Nevada-Reno,

University of Minnesota, and the Shirakawa Institute of Animal Genetics (Japan). This is important because it reduces the number of larger gaps in the bovine chromosomes and gives researchers more choices among markers with a higher probability of finding informative markers in specific populations and families. This has resulted in more successful searches for the chromosomal locations of genomic differences affecting traits that are important for beef production.

To improve the resolution of the human-bovine comparative map, 60 mammary-expressed genes were selected for mapping and markers representing 42 of these genes were positioned on the physical and linkage maps by ARS BARC and MARC scientists, resulting in 61 releases to GenBank. Results of this research provide map information for genes relevant to mammary gland development and function and may aid in the identification of candidate genes affecting economically important traits in dairy cattle production. Previously, the role of BARC scientists on an IFAFS consortium grant (led by Michigan State University) was to map 200 differentially expressed genes onto the BAC map. Probes for 31 genes and markers were screened against the BAC libraries, and this data was submitted to the International Bovine BAC Map Consortium (IBBMC). Due to the impending release of the bovine genome draft sequence, the objectives were modified to generate information that would facilitate annotation of the bovine genome, including BAC-end sequencing, full-length insert sequencing of cDNA, SNP discovery and validation from differentially expressed genes, and discovery of small RNA transcripts. Approximately 25,000 BAC ends and 300 full-length cDNA were generated.

ARS MARC scientists, working collaboratively with colleagues around the world, have successfully integrated *in silico* the publicly available information from a number of linkage maps, radiation hybrid maps, and the bovine BAC map to form an integrated physical map of the bovine genome, including over 9,000 individual mapped markers. This map greatly simplifies the process of locating genes and new markers on the bovine genome, allowing “one-stop shopping” for locating features on the bovine genome. Prior to the availability of the integrated map, scientists would locate one feature on one map and another feature on another map and then be required to resort to relatively crude techniques to tie the information together. Scientists are using the integrated map to assemble the upcoming bovine sequence, identify BAC clones containing a gene target, and discover and validate new SNP markers.

The Bovine Genome Project steams forward (5). A major international project has been underway since 2003 to develop an annotated DNA sequence assembly of the bovine genome following leadership of ARS in the National Science and Technology Council’s Interagency Working Group on Domestic Animal Genomics. This project has been led by the research team at Baylor College of Medicine’s Human Genome Sequencing Center in Houston, Texas, with \$53 M in funding provided by an international consortium led by NIH and USDA. The NIH National Human Genome Research Institute committed 50% of the estimated total project costs for the project. Other funding has been provided from USDA (\$10 M from NRI and \$1 M from ARS), the state of Texas (\$8 M), the Kleberg Foundation (\$2 M), Genome Canada (\$5 M), New Zealand (\$1 M), Australia (\$1 M) and U.S. national and state beef councils (\$.8 M).

An international consortium of researchers has been working alongside of the Baylor team to develop and carry out this project, including several key ARS contributions: 1) *Sequencing animal is inbred Hereford female*. The sequencing effort is being carried out on the DNA of an inbred Hereford female from the long-term Line 1 inbreeding and selection experiment at the ARS Fort Keogh Range and Livestock Lab. 2) *ARS researchers at Clay Center, NE have developed a scaffold onto which to assemble the bovine genomic sequence*. 23 million individual sequences (6.2-fold coverage) of the bovine genome had been produced at Baylor as of June 2005 and subsequently placed into 102,467 assembled sequences. These assembled sequences can now be positioned onto individual bovine chromosomes using the new integrated bovine map scaffold permitting the association of sequence differences with economically important traits such as feed efficiency, meat quality, viability, disease resistance and reproductive rate. 3) *Multi-breed DNA panel developed for building of the bovine*

haplotype map. One of the major thrusts of the bovine genome project is to develop a large pool of single nucleotide polymorphisms (SNP) for use in evaluating genetic diversity and in development of whole genome marker-assisted selection genetic improvement programs. ARS scientists have provided the leadership, in collaboration with breed societies around the world, for the development of an International Bovine HapMap Resource Population containing ~500 animals representing 18 breeds to develop and validate more than 20,000 SNP from a larger pool of ~1M putative SNP identified in the project. This will ultimately lead to a haplotype map of the bovine genome. 4) *DNA resources developed for large panel of full-length complementary DNAs (cDNAs)*. ARS scientists at Miles City, Montana collected tissues from animals related to L1 Dominette 01449, the base DNA source for the bovine genome sequence. A wide range of tissues were collected and are being used for construction and sequencing of a number of cDNA libraries in collaboration with Genome Canada and others. Additionally, ARS scientists completed full clone sequencing for 954 clones predicted to contain the complete protein coding sequence of the gene, annotated the gene for its predicted protein sequence and, where possible, gene function, and deposited the DNA and protein sequences in the public database of sequence information at the NCBI, with more than 350 having so far provided the basis for annotated genes in the draft bovine genome.

As of December 2005, sequencing was completed at BCM and the final assembly and annotation was underway for publication in early 2006. This well-coordinated effort is a monumental milestone in the history of bovine research.

Characterization of previously identified bovine QTL requires moving from QTL detection in historic populations to implementation of marker-assisted selection (MAS) in contemporary commercial populations (6). This process requires the development of new markers from more detailed genome maps, larger pedigrees with complex family relationships, and more robust and sophisticated statistical algorithms that utilize all the genetic information available in the various complex pedigrees. Two specific cases of ARS work with applications to dairy cattle genetic improvement highlight this approach.

For investigations into multiple dairy QTL on bovine chromosome 6 (BTA6), ARS scientists built the most detailed linkage map of BTA6 using resources available through the Cooperative Dairy DNA Repository (CDDR). This allowed construction of a haplotype map of the QTL containing regions, allowing inheritance of specific haplotypes across the entire Holstein population to be followed. Development of this map included marker development from a chromosome-specific library of BTA6. Ultimately, a mutation, which is a candidate underlying a QTL for protein percentage on BTA6, was found in the *osteopontin* gene promoter in complete linkage disequilibrium across multiple sire families. A provisional patent application for using this polymorphism information in MAS was submitted.

For the dairy form QTL on chromosome 27 (BTA27), a more detailed comparative map of BTA27 was constructed in relation to the human genome draft for the purpose of fine mapping. The results of this map suggested that the dairy form QTL detected near the telomere corresponded to regions of conserved synteny between BTA27 and human chromosome 3 (HSA3). Detection of this previously unknown region of conserved synteny improved the BAC map and allowed development of previously unavailable markers distal to the dairy form QTL located at the telomere of BTA27. The markers developed from these findings are being used to further resolve dairy form QTL. Now, the same strategy used to pinpoint the QTL for protein percentage on chromosome 6, combined with information from the draft assembly of the bovine genome, is being used to find mutations underlying QTL on BTA27.

Swine physical map resources completed (7). To facilitate the use of high-throughput automated genotyping systems for swine research, SNP markers located across the genome needed to be identified. At the same time, swine researchers were hindered by the poor resolution of the pig-human

comparative map. Thus, a study to develop SNP markers from expressed sequence tags was initiated to simultaneously address both needs at MARC. A SNP linkage/comparative map that spans the genome has now been completed with over 1,000 loci mapped (approximately 1,500 SNP markers). The porcine SNP markers made available represent the fifth largest collection in mammals at this time. This resource allows scientists or industry partners to test and use these markers throughout the world. Information from these SNPs is being used in the U.S. and abroad to develop paternity, identity, and product trace-back sets of markers that will serve as efficient and durable markers for the needs of the swine industry.

A complete physical map of the porcine genome enables researchers to rapidly develop genetic markers in specific regions of the genome and determine the complete sequence of a gene for the pig. To develop this resource, an international collaboration was developed between the BBSRC, the University of Illinois, INRA and ARS. The laboratory work was primarily conducted at the Sanger Genome Sequencing Centre (Hinxton, UK). A genome-wide BAC map was developed based on fingerprint data from over 265,000 BAC clones and BAC end-sequence data and these data have been made publicly available to all researchers (based upon DNA of a Duroc boar). This resource was developed with the goal in mind of a minimal tiling path for BAC-based sequencing of the swine genome.

International Swine Genome Sequencing Consortium (ISGSC) successful in launching sequencing of the swine genome (8). The International Swine Genome Sequencing Consortium has been working for the past two years to bring together the funding to launch the swine genome sequencing project. The ISGSC consists of members representing ARS, INRA, BBSRC, Japanese National Institute of Agricultural Sciences, Korean Livestock Institute, Sino-Danish Swine Genome Project, University of Illinois, Iowa State University, North Carolina State University, and the US National Pork Board. The USDA National Research Initiative announced in January 2006 that \$10 M in funding will be provided to develop a 3-fold BAC-skim coverage of the swine genome at the UK's Sanger Center in collaboration with the University of Illinois, Iowa State University, and ARS MARC, based upon the minimum tiling path of clones from the swine BAC map. Other international contributions from INRA, Genoscope, BBSRC, the European Commission, and the Korean Livestock Institute are leveraging these funds to ultimately result in 6-fold draft sequence coverage of the genome. This project will be conducted during 2006 and 2007 and should yield a similar set of tools to what has been achieved previously from the chicken and bovine sequencing projects.

Problem Area IVB – Genotyping Systems

Problem Statement: Commercial populations of livestock and poultry have genes affecting economically important traits. However, labor and other cost constraints limit the ability to collect the necessary genotypes. High-throughput genotyping systems need to be developed to permit the collection of genotypic data for loci spanning the genome.

Committed Goal: Reduced cost and increased rate of genotyping.

Planned Approach: Evaluate and develop genetic marker analysis systems that are highly automated and produce reliable results for single nucleotide polymorphisms and microsatellites.

Expected Outcomes: Genotyping systems that can be used to collect large numbers of genotypes per animal in a timely and economical manner.

Engaged ARS Locations: Beltsville, MD; Clay Center, NE .

Selected Accomplishments:

Development of high-throughput SNP assay genotyping chips (1). One of the major products of the bovine genome sequencing project (BGSP) has been the development of SNP-based genotyping assays. In the BGSP, light sequencing (~.2-X) was conducted on animals representing the Angus, Brahman, Holstein, Jersey, Limousin, and Norwegian Red breeds to identify a large pool of ~1M putative SNP. A consortium of researchers, led by ARS, has been working to develop and validate a panel of these SNP to allow determination of genetic diversity of the world-wide cattle population, develop a haplotype map of the bovine genome, and develop high-throughput genotyping chips for use in research programs around the world. To start this process, ARS scientists tested the first batch of 100 predicted SNP by developing genotyping assays on a Sequenom MassArray system followed by genotyping an ARS panel of 96 animals representing 15 beef breeds and Holstein developed previously for animal identification and traceability purposes. Results validated the success of the SNP discovery effort (over 90% of the putative SNP validated), which supported the extension of this approach to identify >30,000 SNP across the genome. Subsequently, a genotyping chip containing 9,800 SNP for cattle genome analysis was produced by ParAllele Biosciences and has been used to genotype the International Bovine HapMap Resource Population Panel in collaboration with the BGSP at Baylor. Collaborators from Genome Canada, the University of Illinois, and Norway are developing an additional set of assays to reach the goal of 30,000 SNP.

Problem Area IVC – Tools and Reagents

Problem Statement: Genomic sequencing provides information needed to obtain rapid gains in agricultural productivity essential to feeding an ever-increasing population. This information, coupled with understanding of gene expression, leads to further understanding biological processes of food production. Nucleotide sequences from normalized cDNA libraries can be used to generate complete profiles of expressed genes for specific tissues and species. Microarrays of genes (cDNA clones) provide a means to study expression of thousands of genes simultaneously, elucidate the function of unknown genes, and identify important regulatory genes.

Committed Goals: 1. Construct and sequence normalized cDNA libraries of expressed genes from various tissues and different developmental time points. 2. Develop cDNA microarray technologies, to provide reliable gene expression profiling.

Planned Approaches: 1. Construct and normalize cDNA libraries from a multitude of tissues and developmental time-points. 2. For each library, sequence clones from both the 5' and 3' ends until the level of redundancy inhibits the usefulness of further sequencing. 3. Evaluate microarraying systems to determine the most useful and economical systems available.

Expected Outcomes: 1. Identification of clones and cDNA sequences for genes expressed in food animal species. 2. Microarrays that can be used for gene expression experiments.

Engaged ARS Locations: Ames, IA; Athens, GA; Beltsville, MD; Clay Center, NE; East Lansing, MI.

Selected Accomplishments:

Understanding how variation in specific Marek's disease virus (MDV) genes influences the immune system is of fundamental importance for animal health and production (1). ARS scientists developed and completely sequenced (175 kb) a disease-causing clone of the MDV genome. This infectious clone, which can be easily manipulated, enables identification of critical virus-host interactions and development of diagnostic reagents. Utilization of the cloned virus will lead to the identification of immunological pathways and genes that promote disease resistance, and may provide a method for developing efficacious vaccines.

Expressed sequence tags (EST) developed from chicken tissues (2). Small intestine ESTs were developed from normalized cDNA libraries by ARS scientists. Of 36,061 clones analyzed, the average insert size was determined to be 1.56 kb, with an average sequence length of 418 bases. Assembly of the ESTs resulted in 4,177 multi-component contigs and 19,741 singleton contigs. 14,374 high quality EST's of greater than 100 base pairs were submitted to the GenBank dBEST databases. Approximately 17% of these expressed sequences have not been previously described and may represent genes unique to the small intestine.

The chicken multi-tissue (brain, ultimobranchial gland, parathyroid gland, cecal tonsil, primordial germ cells) cDNA library has also been sequenced. All 13 plates of the non-normalized ESTs, and 57 plates of the normalized ESTs have gone through the in-house processing software and 21,712 EST sequences passed Genbank submission criteria and have been deposited. Approximately 65 - 70% of these sequences were unique elements. The analysis and screening of the EST sequence data against known genes is ongoing. The analysis and screening phase of this library was scheduled for completion by the end of calendar year 2005. These sequence data will be very useful in annotation of the chicken genome and in the identification of new genes that are associated with economically important production traits.

ARS leads the deposit of bovine and swine EST in to NCBI's GenBank dbEST database (3). ARS data provided the underpinnings for creation of the EST-based linkage map, the radiation hybrid-based physical maps, and the comprehensive map incorporating both linkage and radiation hybrid data that is being used as a framework for assembly of the bovine genome sequence. ARS developed amplification primers by alignment of porcine and bovine EST sequence with the human genome as guide, amplified and sequenced the resulting STS in a panel of animals to identify SNP, genotyped the polymorphisms in the mapping populations to determine their map position in the genomes, and subsequently added 1,500 gene-targeted markers to the genome maps of cattle and swine. Collaboration with the Roslin Institute created a genome-wide radiation hybrid map based on the STS generated during the EST mapping project. A subset of EST clones whose 5' end sequence indicated they were likely to have unique 3' ends were rearranged and sequenced from the 3' end to provide the essential data for construction of cattle gene microarrays capable of monitoring gene expression. These data formed an important basis of an Affymetrix oligo-based microarray, as well as the foundation of a "long oligo" array being constructed by a consortium of universities. The 5' EST sequence data was also used to predict a set of clones likely to contain the complete protein coding region of the gene, which were then rearranged, and collected the complete insert sequence to support prediction of protein sequence for proteomics in cattle. The first 954 of these full-insert sequences have been deposited in GenBank, and an additional >500 are undergoing the annotation process.

First-pass sequence data from two porcine cDNA libraries (MARC 1PIG, MARC 2PIG) have been completed for all available clones. Two more recent libraries (MARC 3PIG, MARC 4PIG) are still being sequenced. ARS has contributed over 33% (about 140,000 ESTs) of all the sequence data available in the public domain for the pig. A porcine gene index representing 104,327 unique gene sequences has been constructed in collaboration with TIGR (www.tigr.org/tdb/ssgi/) using all publicly available data. These data have a significant worldwide impact on research efforts in the pig by facilitating studies in gene expression and efforts to screen populations for important genetic variation. To date, over 6,250 SNP have been discovered within 1,590 amplicons. The ARS porcine reference population has been genotyped for more than 1,640 of these SNP for 1,190 of these expressed genes.

Normalized bovine cDNA library having impact (4). The development of a cDNA library resource has been completed to facilitate the search for specific metabolic pathways, transporters, growth factor receptors, and growth factors which have effects on ruminant visceral energy and protein metabolism. A novel normalized cDNA library has been synthesized from lactating dairy cow and neonatal calf intestine

to facilitate gene discovery and delineate critical pathways relating to nutrient use and visceral tissue growth. This library (BARC 8BOV) has been synthesized, sequenced and characterized and is serving as an indispensable resource in the study of genetic regulation of ruminant nutrient metabolism and growth. This resource is having a direct impact as evidenced by 19,110 new EST sequences deposited in GenBank and by ongoing external interest by cooperators requesting these materials. A total of 1,123 sequence elements from these EST represent genes encoding proteins in other animal systems that are now represented in bovine. This resource has facilitated the development of second generation tools (bmet microarray), an in house Nimblegen array, and commercially available arrays via database accession.

Development of bovine microarrays for transcriptome profiling of mammary tissues (5). Profiling of gene expression within the bovine mammary gland requires an experimental resource that can track changes in thousands of bovine genes simultaneously. Development of this resource is an ongoing process involving continued refinement. In 2003 ARS researchers and the DNA Array Unit in the NIH's National Institute on Aging generated nylon-based microarrays containing over 9,400 cDNA sequences corresponding to more than 5,000 different genes based upon the ARS mammary EST library. In 2005, a collaboration with Nimblegen resulted in production of an oligo-based array. The array contains approximately 380,000 oligonucleotides (60mer) representing >53,000 potential genetic sequences. These arrays will be a central experimental resource for functional genomics research at ARS, and should help identify changes in gene expression that impact mammary gland development, lactation persistency and mastitis resistance. The arrays are also being used in studies with other laboratories and will have potential impact on functional genomic research in cattle.

SAGE libraries produced from bovine mammary tissues (6). There is a specific need to characterize gene expression in the mammary gland during lactation. However, the disproportionate abundance of milk protein transcripts during lactation presents major challenges in quantifying expression of other transcripts that are of interest. Serial analysis of gene expression (SAGE) is a relatively new methodology that holds promise for transcript profiling under these conditions. ARS has produced two SAGE libraries using mammary tissues collected from lactating dairy cows milked either 2x/day or 4x/day to determine whether SAGE is a feasible approach to study gene expression in lactating mammary gland and to identify the genes responsible for enhanced milk yield observed in animals under more frequent milking regimens. Results indicate that SAGE is a useful approach to measure gene expression during lactation and to identify novel gene transcripts. Additional analyses to identify potential genes regulating enhanced milk production are underway.

DNA panel improves the accuracy of prion genotyping in sheep (7). Variation in the prion gene is associated with susceptibility and resistance to scrapie, a neurological disease of sheep that is similar to BSE in cattle. ARS scientists discovered new genetic variation in the prion gene of cattle, sheep, and deer, improving the effectiveness of diagnostic tests. Also, a control panel of sheep DNA was created to increase the accuracy of prion genotyping by research and commercial laboratories. ARS scientists created a control DNA panel from sheep representing each of 15 prion genotypes associated with susceptibility and resistance to scrapie. The control DNA panel is used to detect genotyping errors and to improve the quality of genetic information. This valuable resource is helping producers in the United States and other countries to correctly select for genetic resistance to scrapie and to achieve the industry goal of eradicating scrapie.

Problem Area IVD – Genomic Enhancement Systems

Problem Statement: Developing effective and efficient methods to make precise genome modification is essential for improving productivity, health, and welfare of food animal species beyond levels that can be obtained by selection and increasing genetic diversity. Methods include inactivation, modification or

replacement of native genes, geneclusters or chromosomes and addition of native, foreign or synthetic genes to the genome of food animals.

Committed Goals: 1. Develop methods to extend the life of food animal somatic cells during in vitro culture. 2. Develop effective somatic cell nuclear transfer technology for food animals. 3. Develop alternative homologous recombination procedures for food animals.

Planned Approach: Develop methods to effectively incorporate recombinant DNA into the genome of food animal cells, which can then be used for cloning of live offspring.

Expected Outcomes: Techniques for precise gene insertion that can be routinely used to study the function of genes.

Engaged ARS Locations: Beltsville, MD; East Lansing, MI.

Selected Accomplishments:

Bovine trophectoderm cell lines produced as in vitro models (1). ARS scientists created many new bovine trophectoderm cell lines from NT, IVP, parthenogenetic, and in vivo embryos that were subsequently characterized by 2-D gel electrophoresis and MALDI-TOF mass spectrophotometric analysis. Most of the cell lines were also assayed for production of interferon-tau. All of these cell lines are potentially useful in vitro models for comparing differences between nuclear cloned embryos and normal embryos produced from the union of egg and sperm. Also, the cell lines are all potentially long lived, if not immortal, and, therefore, may be useful as 'nucleus donor' cells in the NT technique. They may also be useful reagents from which to attempt the derivation of bovine embryonic stem (ES) cells since it is possible that with the genetic manipulation of a few or even one transcription factor (e.g., Oct-4) the trophectoderm or endoderm could be converted or 'dedifferentiated' into ES cells.

Less recognition of pregnancy signal protein produced in nuclear transfer derived embryos (2). ARS scientists confirmed that primary nuclear transfer (NT) bovine embryo-derived trophectoderm cell cultures produce less interferon-tau, the 'recognition of pregnancy' signal protein of the cow, compared to in vitro-produced embryo-derived cultures. The lower expression of interferon-tau in bovine NT embryos may be useful as a marker of successful 'reprogramming' in nuclear cloned embryos, i.e., NT embryos exhibiting a higher level of interferon-tau expression may be healthier than NT embryos that produce a low level of interferon-tau.

Problem Area IVE – Bioinformatics and Statistical Analysis Tools

Problem Statement: Developments of bioinformatic tools has lagged behind development of genomic laboratory procedures. Current genomic tools can generate data much faster than can be analyzed despite faster computer processors. Software needs to be developed that is capable of searching the available genome databases for comparative information. Efficient data processing will provide investigators with rapid results, which permit rapid formulation of hypothesis for the next set of experiments to hasten scientific progress. Statistical analysis software needs to be developed to interpret results from genomic and proteomic experiments and the collective results from many different types of data.

Committed Goals: 1. Databases of genomic and proteomic information. 2. Computer software for efficient manipulation and analysis of genomic and proteomic data.

Planned Approaches: 1. Develop database algorithms for storing genomic data. 2. Develop software to search and retrieve data from public databases via the internet. 3. Improve analysis to summarize genomic sequence, linkage, gene expression, and other related data.

Expected Outcome: Publicly available and highly interactive data management and manipulation tools to expedite genomic research.

Engaged ARS Locations: Beltsville, MD; Clay Center, NE; East Lansing, MI.

Selected Accomplishments:

Bioinformatics capacity significantly enhanced at the ARS US Meat Animal Research Center (1).

Software to design primers to amplify introns in genes based on aligning livestock expressed sequence tags (EST) with human genomic sequence was developed by ARS scientists at MARC. This software was employed to genetically map approximately 2,000 genes in cattle and swine. These mapped genes helped resolve the comparative maps among human, bovine and swine. The comparative maps facilitate the identification of livestock (bovine or swine) genes using human sequence and annotation.

Additional software was developed to link function to expressed sequences using Gene Ontology. This software facilitates annotating the bovine genome. Several scientists have used this software to identify positional candidate genes for quantitative trait loci.

Bioinformatics software to identify full-length cDNA clones by comparing bovine expressed sequence tags to human sequence was also developed at MARC with additional software developed to coordinate full-length cDNA sequencing. Using this software, ARS collaborators obtained full-length sequence for over 1,000 bovine cDNA clones. The NIH's National Center for Biotechnology Information (NCBI) is using this information to annotate genes on the bovine genome sequence.

Software that integrates single nucleotide polymorphism (SNP) discovery with a genomics database was built. The software has been used to map several thousand genes and chromosomal loci in cattle, swine and sheep. The software was used by collaborators to identify most of the SNP segregating in a 20 Kb chromosomal region including the prion gene. An abnormal isoform of the prion protein has been implicated in Bovine Spongiform Encephalopathy (BSE). The prion SNP discovery project was a monumental task, which would not have been feasible without the software.

Bioinformatics techniques were used with publicly available sequence to identify twenty-seven novel porcine repetitive elements. Filtering repetitive elements is fundamental to sequence analysis making this work highly relevant to scientists using swine sequence. In addition, repetitive elements have been implicated in gene expression, imprinting, epigenetics and disease.

Statistical analysis software tools developed (2). A software package (GenoProb) that extracts segregation information from genetic marker data in complex pedigrees with marker data on only a fraction of the individuals was developed. The method developed should serve as a useful prototype for the integration of DNA markers into the beef cattle industry's national genetic evaluation system. GenoProb was distributed to 52 people who requested it. Requests were received from research institutions, universities, DNA testing companies, cattle, swine, and plant breeding companies, and a breed association, from Argentina, Australia, Austria, Belgium, Brazil, Denmark, Hungary, the Netherlands, New Zealand, Slovenia, Thailand, the United Kingdom, and the U.S.

An improved statistical analysis for identifying and using chromosome locations of genetic effects was developed. There are several advantages to treating chromosomal locations of genetic effects as random (as opposed to fixed) effects in statistical analyses for identifying chromosomal locations and for genetic evaluation, but this approach is computationally demanding unless marker data is present for all

animals in the pedigree. An algorithm that makes this analysis feasible when marker data is missing was developed and was implemented in software. An interface between this software (MTDFREML) to GenoProb, the software that extracts segregation information from genetic marker data, was added. This software combination is available to the public. It has been requested by researchers working in QTL detection and will be useful for marker-assisted selection. A manuscript describing the algorithm has been downloaded 114 times.

Bioinformatics resources developed at the ARS Beltsville Agricultural Research Center (3). Prior to the initiation of this project, inventory, pedigree, genotypic, and phenotypic data in use by ARS researchers were stored in disparate databases using Microsoft Access. It has been a major effort to streamline and simplify the database structure.

Integration of Genoprob into routine data analysis has progressed. Genoprob allows for sophisticated inference of probability of inheritance of genomic regions. This integration has allowed improved detection of genotyping errors, pedigree mistakes, and poorly behaved markers. The inheritance probabilities are also being used for QTL mapping and complex pedigrees, where traditional mapping techniques are incapable of adequately modeling the complexity of the relationships.

A software package called EST-PAGE was developed and released. This package allows researchers to easily process EST data including: base calling, vector trimming, screening for contaminants and low complexity regions, and dbEST submission to NCBI. This software is being made available as an open source project. As a result, over 25 groups around the world have requested and received source code for EST-PAGE.

A software package called SNP-PHAGE was developed and is being released. This package allows researchers to easily process DNA sequence data for polymorphism discovery and submission to the public database (GenBank's dbSNP). This software has a web interface for data analysis and visualization. This software is being used for analyzing soybean and bovine sequences to discover more than 10,000 SNP. A manuscript describing this application is under preparation. This software is being made available as an open source project. The development of these turnkey packages will enable small genomics groups without bioinformatics support to process data rapidly, and most importantly, to submit data to the public databases for widespread use.

Machine learning was implemented in a new software application package SNP-PHAGE-ML to improve the accuracy of the polymorphisms prediction accuracy. A five-fold increase in success rate of genetic marker discovery was achieved from this software. A manuscript describing this application has been submitted for publication and is currently under review.

Complete genomic variation analysis was completed comparing cattle and other mammals. Neutral mutation rates within each lineage were estimated; cattle-human-dog orthologous fragments were compared to reveal the change of the genome sizes and measured the potential contribution from transposable elements.

A robust pipeline to enable rapid, routine analyses of microarray data is well along in development. Microarray data analysis tools were created to optimally analyze both candidate-gene cDNA and synthesized long-oligo microarray data sets. The first phase of this pipeline is to ensure that only high-quality data are analyzed using extensive quality-control characterization of the data. Next, using heirschel mixed model in SAS, data are normalized to account for such things as slide or probe design. Finally, complex experimental designs can be accommodated using a sophisticated set of SAS programs.

An alternative strategy for gene expression profiling that has been widely used in the ARS Beltsville labs is SAGE, or serial array of gene expression. SAGE databases have been made public via the Beltsville Animal and Natural Resources Institute web site (including clickwrap license agreement for access) as well as submitted to the GEO database at NCBI. In addition, analysis and annotation tools have been developed specifically for SAGE data, including the customized DNA sequence processing pipeline, and gene annotation and gene ontology annotation tools.

RESEARCH COMPONENT V: NUTRIENT INTAKE AND UTILIZATION

Feed represents 50 to 60 percent of the total cost of food animal production. For ruminants 60 to 80 percent of life-cycle feed is stored or grazed forages. Fiber serves an important role in protecting animal health and fiber indigestibility is an important factor in total manure output. Feeding and nutritional regulation of cells and organs jointly affect every aspect of livestock and poultry production. Agricultural industries are challenged to efficiently produce livestock products and to balance growth, feed consumption, and management of waste products. Appropriate nutrition may stabilize animal health, reduce incidences of health-related production losses, and increase healthiness of food products from animals. Introduction of new genotypes may require the development of new feeding strategies. Conversely, specific nutrients may facilitate direct regulation of gene processes.

Vision Statement: Nutritionally efficient livestock and poultry raised in an economically profitable and environmentally sound manner.

Mission Statement: We seek to develop cost-effective strategies that maximize nutrient use by livestock and poultry and to reduce environmental impacts of animal agriculture.

Impact: Efficient use of natural resources and harvested feed in providing healthy, abundant, and nutritious food products from livestock and poultry raised in production systems that have reduced environmental impact of associated waste products.

Linkages: USDA-ARS National Programs: 103 Animal Health; 105 Animal Well-Being and Stress Control Systems; 205 Rangeland, Pastures, and Forages; 206 Manure and Byproduct Utilization; and 207 Integrated Agricultural Systems.

Other Agencies and Departments: USDA-CSREES, Cornell University, Iowa State University, Langston University, Montana State University, and the Universities of Florida, Georgia, Idaho, Maryland, Nebraska, Oklahoma, Wisconsin, Wyoming.

Private Sector: Grow Valley Livestock Coop., Inc., Continental Grain Company, Delmarva Poultry Industry, Inc., Cargill Hybrid Seed, Mycogen Corporation, Pioneer Hi-bred International, Inc., W-L Research Incorporated and ChemGon, Inc.

Problem Area VA –Regulation of Nutrient Gene Function

Problem Statement: Livestock and poultry improvement programs are changing genetic merit for production. Nutrient requirements must be determined and met to realize the improved genetic potentials. Development of feeding systems that capitalize on genetic gains depends on underlying physiological and metabolic processes. Producers must fine-tune delivery of nutrients to optimize production while minimizing nutrient losses to the environment. Therefore, a comprehensive understanding of the metabolic or physiological functions that limit production potential is required. Regulatory genes responsible for limiting metabolism or expression of production potential must be specifically targeted.

Committed Goals: 1. Elucidate regulatory steps and their genetic controls that inhibit maximal performance. 2. Alleviate metabolic or physiological limitations restricting performance. 3. Optimize

animal nutrient efficiency to maximize conversion of nutrients to food products and balance environmental impacts with costs of production.

Planned Approaches: 1. Determine factors limiting maximal animal responses and identify metabolic or physiological functions controlling nutrient use. 2. Assess differential expression of relevant animal and microbial peptides in response to alterations in nutritional or physiological status. 3. Identify animal models to reveal the extent of metabolic and physiological capacity to altered nutrition. 4. Use *in vitro* test systems to model tissue-specific responses to nutrient modulated expression of metabolically important genes and biochemical pathways. 5. Identify mechanisms by which nutrient components affect or regulate genes involved with accretion of fat and lean or composition of milk. 6. Explore the genetic basis for nitrogen and phosphorus metabolism and the potential to manipulate intake/output relationships in N and P metabolism.

Expected Outcomes: 1. Improved nutrient management to enhance animal performance of existing genetic potential. 2. Improved genetic selection targeting specific metabolic or physiological limitations to production.

Engaged ARS Locations: Beltsville, MD; Clay Center, NE; El Reno, OK; Miles City, MT.

Selected Accomplishments:

Forage utilization of cattle was determined with various supplements (1). The patterns of nutrient absorption and utilization when low-quality forage was supplemented with: 1) energy, 2) nitrogen available for rumen digestion, and/or 3) direct provision of dietary protein to the animal were determined. The studies indicated that supplementation with energy alone may have negative effects on the animal's nutritive status and that supplementation that provides a mixture of energy and protein that is both available to ruminal microbial growth and directly to the animal provides the most favorable patterns of nutrient absorption and utilization. These findings will aid development of improved supplements for ruminants grazing low-quality forages.

Matching nitrogen source to diet improves nitrogen utilization (2). The relative contribution of nitrogen that is transported from blood into the digestive tract to meet a sheep's nitrogen requirement differs depending on the animal's diet. Sheep eating high grain diets were shown to not benefit from additional nitrogen transport back into the digestive tract, whereas, those on low quality forage did benefit. Also, sheep on low quality forages had greater efficiency of nitrogen retention when some of the nitrogen was provided as protein. These findings will be important in developing new recommendations for feeding nitrogen (form and amount) to ruminants.

Genetics of compensatory growth and feed efficiency explored (3). Growing beef steers were used to evaluate changes in gene expression in the liver in response to feed restriction and re-feeding to identify genetic mechanisms regulating visceral growth and metabolism of cattle. The goal of the study was to identify gene pathways controlling compensatory growth and improved feed efficiency. As expected, improved feed:gain ratios were observed in animals experiencing a period of dietary restriction. Currently, a microarray containing oligonucleotides representing over 53,000 potential gene transcripts produced by ARS in collaboration with Nimblegen is being used to characterize these changes in hepatic gene expression over time. Analyses to identify potential genes regulating enhanced feed efficiency are ongoing and results from this work should identify gene targets for selection to improve feed efficiency.

Influence of genotype and diet on steer performance, manure, and odor (4). Diet × genotype (proportion Brahman) interaction in terms of feed intake, feed utilization, and performance of steers fed

ground bromegrass hay or corn silage-corn diet and compensatory performance during finishing was evaluated. *Bos indicus* crosses consumed less feed, grew slower, but had similar efficiencies as *Bos taurus*. No interactions were observed. This work revealed that the composition of feed influences the composition of manure and production of odor compounds produced during manure fermentation.

Genes that regulate appetite and energy balance in poultry (5). There is currently very little information concerning the genes that are responsible for regulating appetite and energy balance in domestic poultry. ARS researchers have successfully identified and cloned portions of the ghrelin gene in both chickens and turkeys and have begun to study its expression under different physiological conditions. The chicken ghrelin gene, which exhibits a high degree of similarity with mammalian ghrelin genes, was shown to be expressed in stomach (proventriculus) tissue from broiler chickens during periods of feed deprivation and refeeding and from birds subjected to an experimental infection with coccidiosis. This work provides new insight into the structure of the avian ghrelin gene and suggests a potential functional role for this gene in regulating feed intake in chickens and turkeys.

Methionine deficiency shown to alter important growth regulators in chickens (6). In regions where soybeans are the primary proteinaceous component of poultry rations, methionine is the first limiting essential amino acid, and if not supplemented in the diet will result in severe growth deficiencies. Research was conducted to evaluate the effect of a methionine deficiency (moderate to severe) on the chicken endocrine system and other growth parameters. Plasma concentrations of insulin-like growth factors 1 and 2 and the thyroid hormone, triiodothyronine (regulators of growth and metabolism) were significantly lower in chicks fed methionine deficient diets. This work has shown that a deficit of dietary methionine alters the secretion and metabolism of important regulators of growth and development and that dietary methionine must be provided to ensure maximized phenotypic expression.

Role of enzyme complex in energy balance elucidated in poultry (7). AMP-activated protein kinase (AMPK) is an enzyme complex that plays a key role in sensing cellular energy (AMP/ATP) levels, maintaining intracellular energy homeostasis and, on the whole animal level, in regulating energy balance and food intake. In general, AMPK acts to increase cellular energy levels by reducing the activity of ATP-utilizing metabolic pathways and increasing those that generate ATP. Since there have been no prior studies of AMPK in birds, the objective of this work was to identify and characterize the AMPK pathway in chickens. ARS scientists have identified seven distinct chicken AMPK gene homologues for alpha, beta and gamma subunits and studied their expression in different tissues. The expression of these genes confirmed, for the first time, the existence of a functional AMPK pathway in chickens and indicated that AMPK is likely to be a master cellular energy sensor/regulator in poultry. These findings provide new information related to the regulation of feed intake, energy balance and body weight in chickens at the molecular level.

Control points to alter fat deposition studied in aves (8). Unlike mammals, intermediary metabolism of aves is highly regulated by the thyroid axis, which is, in turn influenced by the nutritional status. Research was conducted to determine if thyroid hormone ablation and subsequent hormone repletion would alter the expression of genes controlling lipid deposition in the broiler. This work revealed that certain control points in metabolism can be used to both limit excess lipid accretion in the broiler and to potentially save feed costs.

Problem Area VB – Interactions Affecting Reproduction

Problem Statement: Reproductive processes are affected by numerous nutritional factors such as diet composition, regulatory metabolic hormones, and body composition. Changes in diet are detected by higher brain centers modulating pituitary hormone secretions and impacting reproductive function. Knowledge of cell-to-cell interactions affecting gonadal and uterine function, as modified by nutrient status and body composition, is needed to refine feeding systems and aid in cost effective management

of reproduction. Immune system activity is also linked to energy balance and reproductive efficiency. Optimizing reproduction, immune system function, and disease resistance requires understanding how the basic regulatory axes are influenced by various nutritional factors.

Committed Goals: 1. New nutritional management systems to maximize number of efficiently produced and marketable offspring at appropriate endpoints. 2. Optimal nutritional strategies for developing replacement livestock and poultry breeding stocks.

Planned Approaches: 1. Determine requirements and quantify effects of nutrients such as vitamins, trace minerals and amino acids on components of reproductive efficiency. 2. Determine nutritional effects on neuro-endocrine pathways regulating gonadal function and behavior. 3. Determine the impact of prepubertal and postpartum nutritional strategies and manage systems on reproductive efficiency.

Expected Outcomes: 1. Nutritional management systems that enhance or maximize reproductive efficiency. 2. Increased knowledge of nutritional modulation of cellular functions affecting physiological pathways in livestock and poultry.

Engaged ARS Locations: Athens, GA; Beltsville MD; Brooksville FL; Clay Center, NE; Dubois, ID; El Reno, OK; Miles City, MT.

Selected Accomplishments:

Reduced nutrient intake studied in prepubertal gilts (1). Identifying how level of nutrition contributes to changes in adipocyte function and subsequent fertility are critical for improving overall reproductive performance of the swine breeding herd. Leptin, secreted by fat cells in response to changes in body weight and nutrition, regulates appetite and serves as a metabolic signal that directly affects brain and pituitary tissues to regulate luteinizing hormone (LH) secretion. Reduced nutrient intake in the prepubertal gilt has been shown to alter metabolism yet does not change luteinizing hormone, leptin secretion or fat cell expression of leptin, leptin receptor, fatty acid binding protein and nuclear transcriptions factors. The ability of the prepubertal gilt to maintain energy level in the normal range suggests the prepubertal animal is resistant to moderate reductions in nutrition. This information provides a more detailed understanding of the role of nutrition in modulating adipocyte function and the reproductive axis.

Linkage of seasons, adipocytes and infertility in swine (2). Economic losses associated with seasonal infertility are estimated to be \$150-350 million dollars annually. Identifying critical areas that contribute to seasonal changes in fertility and appetite are critical for improving overall reproductive performance of the breeding herd. A contributing factor to infertility is level of nutrition, reduced body fat and altered adipocyte function. A number of genes and biologically active secreted proteins of adipocytes have been identified such as leptin, many cytokines, IGF system proteins and agouti which have a profound effect on appetite, growth and the reproductive axis. This information will be invaluable in understanding how change in seasons affects reproductive function and feed intake.

Defining selenium metabolism in ewes (3). The effects of reproductive state and organically-bound selenium chemical forms on selenium distribution, selenoprotein activities, and reproductive health were established. Limited information is available defining the metabolic fate of supranutritional selenomethionine (form of selenium in naturally high-selenium feeds) and selenocysteine (form of selenium arriving in the duodenum of ruminants fed selenium salts) in pregnant and lactating animals. A corporation that markets a high selenomethionine yeast product used (unsolicited) this information in a marketing brochure as a “proof of concept” to demonstrate the advantages of selenomethionine products

in enhancing livestock selenium status over other supplemental selenium forms. This research was used to support the use of a product to improve the reproductive health of ewes.

Strategies to reduce use of harvested feeds for producing beef cows evaluated (4). Feeding strategies that allowed body weight loss of cows followed by body weight gain were compared to feeding strategies that maintained body weight. The efficiency of feed utilization for calf production was compared. Neither calf weight at 58 days of age, nor percentage of cows subsequently diagnosed pregnant were adversely affected by weight loss during pregnancy when followed by weight gain during lactation. These results suggest that weight cycling in mature beef cows may be a viable management tool for reducing feed costs.

Problem Area VC – Microbial Effects

Problem Statement: Ruminants rely on diverse microflora to digest feeds. Monogastric animals also host microbial populations in their gut that can influence efficiency of nutrient use. Regional differences in types and availability of dedicated and byproduct feeds and release of new varieties of traditional crop species with improved nutritional properties have increased options for formulating rations to improve production. There is a paucity of information on interactions of these feeds in the upper and lower digestive tract of ruminants and the gastrointestinal tract of monogastric species. Understanding how these interactions affect digestibility, digestion kinetics, and nutrient absorption is important for efficient use of feedstuffs.

Committed Goals: 1. Determine if there is a genetic component to the interaction of an animal with its stable microflora and develop strategies to optimize populations of specific microbial species. 2. Elucidate roles of genetics and environment in determining composition of species of the GI tract or rumen, products of rumen fermentation, and growth efficiency of rumen or GI microorganisms. 3. Determine interactions among rumen microbes and different feeds, particularly byproduct feeds and new genotypes of forage and grain species. 4. Determine efficiency of substrate utilization and microbial protein yield from various feed components under different rumen environmental conditions.

Planned Approaches: 1. Characterize interactions among rumen microbes' fermentation characteristics and efficiencies associated with varying production conditions. 2. Identify gut and rumen microbial species, competitions, and symbioses in animals of varying production levels. 3. Identify individual microbial species instrumental in degradation of recalcitrant plant tissues and in facilitating nutrient passage from the gut. 4. Characterize rumen microflora of high producing dairy and beef cattle and sheep and gut microbes of nonruminant species through successive generations and with different diets. 5. Determine the impact the existing gut microflora on nutrient excretion and how modification of the existing microflora affects nutrient utilization, nutrient excretion, and odors. 6. Develop site-specific models of feed quality incorporating agronomic and animal data.

Expected Outcome: Nutritional systems to enhance and maximize efficiency of nutrient use through optimization of relationships between endogenous gut or rumen microflora and diet.

Engaged ARS Locations: Beltsville, MD; Clay Center, NE; El Reno, OK; Madison, WI; Ithaca, NY; and Ames, IA.

Selected Accomplishments:

Lauric acid supplementation suppresses rumen protozoa (1). Literature reports indicate that reducing protozoa, a major class of microbes found in the cow's rumen, may improve nitrogen utilization. ARS research found that a single daily dose of 160 g of the medium-chain fatty acid lauric acid, when

poured directly into the rumen via rumen cannula, suppressed protozoa by 90-95% within 3 days. This dose also reduced rumen ammonia and milk urea, suggesting improved nitrogen efficiency. However, feeding 160 and 240 g/day of lauric acid reduced the protozoal population by, respectively, only 25 and 30% after 8 weeks. These results indicate that feeding, as opposed to giving a single dose directly into the rumen, will not achieve an effective lauric acid concentration for suppressing rumen protozoa.

Ways to improve stocker calves use of wheat pastures (2). In the southern Great Plains region, annual cool-season grasses, primarily winter wheat, are used to grow and develop stocker calves. Wheat forage contains high concentrations of digestible protein, dry matter and energy and should support excellent body weight gains in stocker calves. Conversely, average daily gains during the first 21 days of wheat pasture grazing is less than anticipated based on forage chemical composition. Initially calves may not eat very much wheat forage or slowly introduce it into their diet because it is too rich in nutrients. During the first 2 to 3 weeks of wheat pasture grazing, stocker calves may experience digestive disorders which reduce forage intake. Direct fed microbial products have the potential to decrease digestive disorders and may improve stocker performance by promoting a healthy GI tract microbial population. ARS research showed that administering a single dose of a commercially available direct fed microbial product to stocker calves prior to grazing spring wheat forage for the first time improved stocker performance. A single dose was not effective in the fall when forage fiber content was the lowest and forage protein content was the highest. In the fall, daily feeding of direct fed microbial product may be needed to overcome digestive disturbances.

Evaluated the in situ method for determining bypass protein (3). The in situ procedure, where fiber bags containing the feeds to be tested are suspended directly in the cow's rumen, is widely used to measure bypass protein (the protein that escapes rumen breakdown and is directly digested in the cow's intestine). The in situ technique was tested on four proteins that had been fed to milking cows in an earlier trial. It was found that the in situ method gave accurate results for two kinds of soybean meal; however, the bypass value of two other major proteins, corn gluten meal and blood meal, was over-estimated. These results indicated that the in situ technique, which was adopted by the National Research Council as its standard method of evaluating feedstuffs, may assign inaccurate protein values to feeds, leading to poor protein nutrition of US dairy cows.

Showed the lower value of rumen-degraded protein from urea relative to true protein (4). Optimizing the amount of protein formed by the rumen microbes is important because this source provides more than half of the protein actually used by the cow. It is widely believed by dairy nutritionists that NPN can replace true protein to meet the requirements of the rumen microbes for rumen-degraded protein (RDP). Experiments were conducted with lactating dairy cows fitted with rumen cannulas to allow omasal sampling to quantify the effect of RDP source on milk production and microbial protein synthesis in the rumen. Diets were composed of alfalfa silage, corn silage, and high moisture corn, typical feeds used for dairy cows in the U.S. and contained a range of levels of RDP added from different true proteins or urea (an NPN source). As urea replaced true protein there was decreased feed intake and milk yield. Replacing RDP from true protein with that from urea also depressed microbial protein formation in the rumen. Replacing true protein with NPN reduces milk production and impairs nitrogen utilization in lactating dairy cows. This information is being used to optimize protein use in lactating dairy cattle diets.

Improved the rumen submodel of the Cornell Net Carbohydrate Protein System (CNCPS) and the Cornell Penn Minor (CPM) System for Evaluating Cattle Diets (5). This computer model allows farmers to formulate cheaper, more efficient rations. The rumen submodel is the most dynamic component of the CNCPS and it accounts for approximately 80% of the total computing time. The National Research Council (NRC) for beef cattle used the CNCPS as its dynamic model (Level 2) and distributed it widely. In 1998, independent consultants hired by the British Government evaluated the CNCPS and compared it to the British System (ME/MP) on farms. Their report indicated that "the CNCPS model predicted milk yield when either energy or protein was limiting to within 2.5 and 5%,

respectively, whereas the ME/MP system over-predicted milk yield from either energy or protein limiting diets by 17 and 46%, respectively. The CNCPS and CPM are widely used in the United States and internationally. The rumen submodel is updated as new knowledge is available. Since 1998 more than 1800 copies of the program have been sold at a cost of \$350 per program. The use of this feeding system has demonstrated improved nutrition for the dairy herd and reduced feed costs.

Elucidated microbial requirements for rumen-degraded protein (6). Feeding rumen-degraded protein (RDP) in excess of the amount needed by the rumen microbes probably is wasted and will be excreted largely as urinary nitrogen and contribute to environmental pollution. Feeding studies defined the in vivo response of rumen microbial protein synthesis to a range of dietary levels of RDP and fermentable energy. The quadratic equation obtained from this work indicated that 10% RDP in the diet gave 82% of the maximal microbial protein yield. Decreasing dietary RDP from about 14 to 10% generally did not affect milk or protein yield but resulted in a 12% reduction in urinary nitrogen excretion. This research defined the forms and levels of protein to simultaneously maximize production and minimize nitrogen losses.

Evaluated different methods for quantifying protein formation in the rumen (7). Accurate measurement of protein formation by the rumen microbes is critical to assessing the influence of dietary factors on nitrogen utilization in dairy cows. Three different procedures were tested for measuring microbial protein synthesis in the rumen: 1) heavy nitrogen (N-15), 2) total purines (present in microbial DNA and RNA), and 3) urinary excretion of purine derivatives. Microbial protein formation in the rumen determined using N-15 was more accurate and precise than the other methods. Microbial protein determined using urinary excretion of purine derivatives was not precise but this method gave the correct comparison among different diets. Measurements made with total purines, the method most widely used by researchers studying protein nutrition in dairy cows, appeared to be less reliable. This knowledge will contribute to improving the use of nitrogen for milk production in dairy cows and reducing diet costs.

Problem Area VD – Minimizing Production Losses

Problem Statement: Under-feeding, over-feeding, or imbalanced nutrition will reduce production efficiency and may make livestock and poultry susceptible to infectious pathogens. Changing genetic potential for production or body composition also may result in imbalanced nutrition and modify the capacity of the immune system to recruit nutrients needed for good health. Animals harboring overt or subclinical disease use a portion of daily intake to combat the disease resulting in inefficient feed conversion to growth, reproduction, milk production, or egg production. With current concerns about antibiotic resistance and chemical residues in food, nutritional modulation of the immune system may be a way to maintain animal health, and maintain a competent immune system through improved nutritional or nutrient-based intervention strategies that lead to specific actions during immune challenge.

Committed Goals: 1. Strategies that appropriately differentiate between feeding for short-term performance and feeding for life-cycle efficiency. 2. Limits and expectations of benefit for specific nutrient-diet modifications in recommending requirements for nutrients to improve animal health and well-being. 3. Establish nutrient requirements and impact of altered nutrition on periodic production processes as related to interactions with neuroendocrine and endocrine immune axes. 4. Discover effects of specific nutrients on immune gene expression, pro- and anti-inflammatory cytokine status, antimicrobial peptide production, and ability to rapidly return to healthful status.

Planned Approaches: 1. Develop species-specific immune cytokines, cytokine antibodies, and cytokine gene probes and assays. 2. Elucidate mechanisms by which nutrients affect immune function and effects of immune response on short- and long-term production. 3. Determine whether genetic selection for increased performance and leanness may adversely alter innate immune defenses. 4. Develop

nutritional strategies for gastrointestinal stabilization to prevent emergence of zoonotic pathogens due to perturbed gut ecology. **5.** Develop nutrient-based strategies to limit consequences of immune system processes resulting in modification of regulatory proteins and DNA damage.

Expected Outcomes: **1.** Nutritional systems that modify immune response and improve animal health. **2.** Reduced risk of pathogenic contamination of animal products. **3.** Improved nutritional management of livestock and poultry.

Engaged ARS Locations: Ames, IA; Beltsville MD; Clay Center, NE.

Selected Accomplishments:

Butyrate implicated in cell proliferation (1). The short chain fatty acid butyrate was shown to have significant effects on the regulation of the proliferation cycle of growing cells in culture, and inclusion of butyrate in the culture resulted in a dose-dependant increase in pathway-specific apoptosis, programmed cell death. Results of experiments suggested that the pattern of volatile fatty acids, especially butyrate, generated in cattle could have a significant impact on the growth and division of epithelial cell populations. This research implies that nutrient management of the generation of butyrate, especially in the gut, may affect aspects of biology ranging from tissue growth to wound repair and recovery from enteric infection.

Feed restriction used to prompt gene expression (2). The effects of feed restriction on the expression of genes involved in fat synthesis and storage in broiler breeder chickens during egg production were studied. When birds were given unrestricted access to feed, fat synthesis and storage were significantly elevated. This led to increased body fat and reduced egg production in breeder hens. Feed restriction as practiced by commercial poultry producers was effective in limiting fat accretion and led to improved egg production. Excess fat production is a necessary component of optimum rates of protein synthesis, but, nonetheless, it is a billion dollar loss item to the poultry industry. Feed restriction, practiced during early life, will ameliorate excess body fat and save the industry \$100M annually.

Urea recycling and metabolism defined (3). In ruminants, the poor efficiency of converting dietary protein into milk or muscle protein results partly from the extensive degradation of protein in the rumen resulting in ammonia absorption and ultimately, excretion of nitrogen in urine. It was not known if a mechanism to synthesize urea from absorbed ammonia existed in ruminant gut tissues, in particular the rumen epithelia. Such a mechanism would be an attractive target pathway for reducing the toxic side-effects of ammonia absorption in the animal and for promoting the local recycling of urea to the rumen for microbial protein synthesis. Using an in vitro isolated cell system and innovative stable isotopic approaches it was demonstrated that ruminant gut tissues have the capability to synthesize urea from substrate intermediates in the ornithine-urea cycle, in particular from arginine degradation by ruminal epithelial cells. Urea synthesis by ruminant gut tissues may be a strategic target to reduce ammonia absorption and improve nitrogen utilization. These results have been basis for a patent application for the use of stimulatory compounds for up-regulation of the argino-succinate synthetase gene.

Defined altered adipose metabolism in cattle (4). Using a carbohydrate infusion model, alterations in adipose metabolism across three primary adipose depots have been defined. Increased efficiency of nutrient use observed by steers fed diets with higher energy densities can be partially explained by alterations in gut tissue metabolism of energy substrates and the subsequent alterations in adipose depot lipogenic rates. Methods to quantify the expression of lipogenic genes present in adipose tissue depots by RT-PCR were developed and used to assess expression of FAS, ACC, Spot-14, SERBP, SERBP-1, SERBP-2, and ChREBP, genes known to be involved in fatty acid metabolism. Demonstration of upregulation of lipogenic metabolism genes as a result postprandial carbohydrate

delivery and identification of Spot-14 as a putative critical controlling element in the response resulted from this research. Deposition and mobilization of adipose depots in the lactating dairy cow and synthesis of adipose tissue in growing steers are critical metabolic processes where efficiency of nutrient use may potentially be manipulated to enhance animal performance and reduce losses to the environment.

Management to reduce fescue toxicosis (5). Fescue toxicosis in cattle consuming *Neotyphodium* infected tall fescue is estimated to cost the livestock industry over \$1 B annually in the U.S. Recent research has demonstrated that management protocols can be used to reduce fescue toxicosis in cattle. Cattle weight gains on endophyte-infected tall fescue are significantly improved when implanted with a progesterone/estradiol benzoate implant, provided forage is not a limiting factor. Further, cattle consuming endophyte-infected tall fescue recover from heat stress related to the intoxication within 4 to 10 days, depending on ambient temperature, following a switch to a clean diet. This would suggest that the effects of the intoxication are not permanent and can be reversed. These findings support that the utilization of endophyte-infected tall fescue in forage-based cattle enterprises should be sustainable if the appropriate combinations of management protocols are implemented. Appropriate management would result in a reduced need to replace robust stands of existing tall fescue.

Problem Area VE – Nutrient Use and Feed Evaluation

Problem Statement: Animal feeding strategies are limited by a lack of understanding of mechanisms involved in plant cell wall lignification that limits fiber digestion, knowledge of nutritional strategies to increase the utilization of nitrogen and carbon and limit the output of excess nitrogen and carbon, and methodology to accurately measure nutrient digestion kinetics and bioavailability. Chemical and biochemical research is needed to understand intrinsic characteristics of fiber limiting digestion and processes plants use to synthesize indigestible compounds. Adverse environmental impacts of nitrogenous wastes emphasize the need to optimize levels of protein fed to livestock.

Committed Goals: 1. Develop strategies that alleviate intrinsic limitations to digestion of feeds, especially fibrous plant cell walls. 2. Develop new methods to determine nutrient bioavailability and protein degradability. 3. Develop dynamic systems for site-specific feed evaluations and feeding recommendations. 4. Develop methods to reduce protein breakdown to nonprotein nitrogen in grazed forages and silages. 5. Optimize nutrient utilization in conversion of feed nutrients into food products while balancing costs of production with environmental impact.

Planned Approaches: 1. Determine the minimum nutrient requirements to support optimum production while minimizing nutrient losses for modern domestic livestock species under different production systems. 2. Determine how the deposition and structure of lignin in the cell walls of forages restrict the potential digestibility of the cell-wall polysaccharides by ruminants. 3. Develop models incorporating feed intake, and digestion and passage kinetics that improve prediction of feed digestibility. 4. Develop systems to measure digestion kinetics more accurately and test and calibrate current and new feed evaluation systems. 5. Develop strategies for improving nitrogen use by synchronizing carbohydrate degradation and protein degradation in the rumen. 6. Develop strategies for improving carbon use by increasing digestibility and utilization of structural and non-structural carbohydrates, and conserving protein in stored forages. 7. Determine how nutrient requirements and excretion can be manipulated through changes in animal physiological processes, diet formulation, environment, and feeding strategies. 8. Develop procedures for use of dietary enzymes, microbial extracts, supplements, and metabolic modifiers to improve nutrient utilization and decrease nutrient excretion. 9. Develop simple, inexpensive, rapid and reliable tests to reliably determine the bioavailability of nutrients in feeds.

Expected Outcomes: 1. More efficient use of forages in ruminant livestock production.
2. Development of nutrient management systems optimizing food production with minimal effect on the environment.

Engaged ARS Locations: Ames, IA; Beltsville, MD; Brooksville, FL; Clay Center, NE; El Reno, OK; Madison, WI; Miles City, MT; St. Paul, MN.

Selected Accomplishments:

Altering lignin synthesis in plants to improve their digestibility (1). Alfalfa is widely used as a fiber and energy source for dairy cow diets. It is limited, however, by poor digestion of the structural carbohydrates (fiber component) limiting the energy recovered from the plant by the dairy cow. Lignin (the glue that holds fiber together) is completely indigestible itself and as a result of how it is made and incorporated into the fiber, the structural carbohydrates also become less digestible. One approach to solving the problem of poor fiber digestibility is to alter the lignin to decrease its negative impact. This work is part of an ongoing research effort of the Consortium for Alfalfa Improvement (a collaboration among the Noble Foundation, Forage Genetics, and the ARS U.S. Dairy Forage Research Center). Collaborators at the Noble Foundation successfully generated alfalfa transgenics that were down-regulated in enzyme C3H, a crucial step of lignin synthesis. Detailed characterization by nuclear magnetic resonance studies showed such plants produced a lignin with dramatic changes in its composition and structure. Over 60% of the lignin is built from a building block that typically is only a minor component in normal lignin. Because lignin is crucial to the plant for strengthening its fibers and water transport, severe down-regulation of C3H depressed plant growth. However, lesser down-regulation still impacts the lignin structure greatly, but restores plants to normal vigor. Such intermediate plants appear to be more digestible. This process provides a potential approach to improving plant fiber utilization in ruminants and provides additional information about how individual enzymes in the lignin pathway contribute to specific types of lignin structures and the impact of altering those structures on their functional roles in plants. This research also has implications for improving the efficiency of use of biomass for production of biofuels.

Expanded on the role of ferulates in cross-linking plant cell wall polymers (2). Ferulates are little bridging molecules that play a huge role in plant cell wall cross-linking. Despite their low concentrations in the wall, they are crucial factors in limiting digestibility in ruminants, and enzymatic degradability in general. In collaborative research ARS has shown previously uncharted cross-linking reactions forming trimeric structures that more extensively cross-link the wall than previously thought. As such actions now must be taken into account by researchers, the finding is stimulating further basic research as well as enhancing the understanding of limitations to digestibility, and ultimately improving our potential to optimize the utilization of valuable cell wall resources. This research is increasing our knowledge on factors limiting biological availability of wall constituents.

Critical reviews and establishment of forage ideotypes (3). U.S. Dairy Forage Research Center projects have progressed to the stage where ARS has been sought out to prepare several critical reviews on cell wall cross-linking and on lignification. Such reviews are valuable in that they synthesize the known findings and evaluate the status and future of research in the area. For example, in a series of three review articles with collaborators in France, we attempted to come up with forage grass ideotypes, i.e., grasses with structural characteristics that make them most amenable to optimal utilization by ruminant animals in producing milk and protein and improving sustainability. This accomplishment will aid coordination and priorities of future research on forage quality.

Forage germplasm identified with reduced ferulates and lignin (4). Smooth brome grass, orchardgrass, and reed canarygrass germplasm has been identified with reduced levels of ferulate cross-linking and Klason lignin in their fiber fraction. Based on previous research, these reductions in cross-

linking and lignin should result in improved fiber digestibility by livestock. The identified germplasm will form the starting point for breeding studies to develop improved grass varieties that incorporate these alterations in fiber and digestibility. These three grasses are very important to dairy, beef, and sheep producers for pasture and hay production in the Northeast and North Central areas of the U.S. Development of commercial varieties of these grasses will require a minimum of 10 years because of their perennial nature.

Low ferulate corn line (5). A mutant corn line was recovered from back-crossed generations that expresses a low-ferulate ester phenotype in seedling leaves. Contrary to expectations, the mutant phenotype appeared to be a dominant trait rather than recessive. If this expression pattern is verified, then future breeding efforts for this trait will be much simpler and cheaper. Based on these results, additional trials are underway to determine if the low ferulate trait results in the expected improved digestibility of the stover portion for corn silage. Such a result would be of substantial benefit to livestock producers because corn silage is among the most important feeds for dairy and beef production, and approximately 10% of all US corn acreage is used for silage.

Determined that many inoculated silages are digested more efficiently than untreated silages (6). Lactic acid bacteria are commonly added to crops when they are stored in a silo to guarantee a good fermentation. Surprisingly, these bacteria sometimes boost milk production in dairy cattle or rate of gain in growing cattle by 5%, much greater than expected. ARS researchers studied how inoculated silages were digested in the rumen, using a laboratory technique that mimics the rumen environment. Compared with untreated silages, it was found that many of the inoculated silages were digested more efficiently - more of the digested portion of the silage ended up in rumen microorganisms and less ended up as gas (mostly carbon dioxide and methane, two greenhouse gases). These improvements in efficiency in the rumen are in line with improvements in milk production and rate of gain that have been observed in cattle experiments. The technique used in this research could be beneficial to inoculant manufacturers in finding even better strains of lactic acid bacteria to improve how cattle digest their feed. These results are being incorporated into the dairy forage expert system to improve the nutritive evaluation of silages.

Factors affecting density and dry matter losses from bag silos were established (7). Bag silos are an increasingly common means of ensiling on dairy farms in the U.S., but relatively little is known about the densities and losses that can be expected from them. In collaboration with the University of Wisconsin, ARS surveyed the filling and emptying of bag silos made at three research farms. Densities ranged from 10-18 lb dry matter per cubic ft. and were influenced primarily by moisture content, how finely the forage was chopped and the operator. Losses of dry matter ranged from 0 to 40%, averaged 14.6%, and were most significantly affected by air temperature at emptying, porosity of the silage, moisture content and how quickly holes in the plastic were patched. Consistent losses of less than 15% are possible if bags are routinely monitored and patched. These results will help farmers already using bag silos to obtain the best results and will help those contemplating bag silos to estimate true costs.

Activity of bacteriocin is shown to be high under ensiling conditions (8). The dairy industry relies heavily upon the use of silage as a feedstuff, but silage preservation is often inadequate to insure quality. One of the critical control points impacting silage quality is the control of detrimental amino acid degrading bacteria during the ensiling process. Previous work by ARS researchers at Ithaca, NY has shown that *Clostridium sporogenes*, the detrimental bacteria, could be inhibited by the bacteriocin bovicin HC5 that is produced by the ruminal bacterium *S. bovis*. Inclusion of *S. bovis* as a silage inoculant has been proposed as an alternative to commonly used Lactobacilli to take advantage of its faster growth rate and ability to cause a more rapid pH decline. The research team has now shown that the activity of bovicin HC5 is at least 10-fold higher at pH of 5.0 as compared to 6.7. These results show that the bacteriocin-producing bacteria has potential to improve silage quality.

Determined optimal levels of dietary crude protein for lactating dairy cows (9). Practical diets fed to lactating dairy cows range from about 16 to more than 20% crude protein. Several lactation trials were conducted that indicated that feeding protein in excess of about 16.5% was wasteful—it did not increase production and led to excessive nitrogen excretion and resulted in nitrogen pollution of water and air. This information is useful for reducing feed cost and reducing nitrogen pollution of the environment.

Identified improved protein supplements for feeding to lactating dairy cows (10). Feed supplements for dairy cows differ greatly in amount of “bypass” protein (i.e., protein that escapes microbial degradation in the rumen and becomes available for direct digestion in the cow’s intestine). Overall digestibilities of the bypass protein from the four proteins differed substantially, averaging 74% (solvent soybean meal), 72% (expeller soybean meal), 81% (blood meal), and 50% (corn gluten meal). Low intestinal digestibility of corn gluten meal, which is regarded by dairy producers as a high bypass protein, will impair its value as a dietary supplement. This information will allow more accurate diet formulations, reducing both costs and losses to the environment.

RESEARCH COMPONENT VI: GROWTH AND DEVELOPMENT

Growth and development impact all components of meat and milk production. Feed consumption is a primary regulator of growth and development. Tissues and biological systems must undergo coordinated developmental changes to form a mature end-product. Growth and development of the fetus and early neonatal animal have lasting effects on health, performance, and productivity. The manner in which muscle and mammary tissue grows and develops dictates the quantity and quality of meat production, as well as the efficiency of how feed energy is converted to a final food product. Growth and development of adipose tissue impact carcass composition, meat quality, and mammary development. Turnover of mammary epithelium during lactation impacts persistency of lactation.

Vision Statement: Optimize growth and development to enhanced production efficiency and product quality.

Mission Statement: We seek to improve conversion of feeds to animal products; increase rate of production of animal products; and improve composition of animal products.

Impact: Livestock and poultry management systems that promote efficient production and products that promote human satisfaction, health, and well-being.

Linkages: USDA-ARS National Programs: 103 Animal Health; 105 Animal Well-Being and Stress Control Systems; 107 Human Nutrition; and 207 Integrated Agricultural Systems.

Other Agencies and Departments: USDA-CSREES, NADDK, Brigham Young University, Purdue University, Michigan State University, and the Universities of Georgia, Idaho, Maryland, Nebraska, and Wyoming.

Private Sector: US-Israel Binational Agriculture Research and Development Fund, Pfizer Animal Health, Alparma, Inc., and GroPep, Inc.

Problem Area VIA – Regulating Feed Intake

Problem of Statement: A major controlling factor of growth across species is feed intake. Feed costs represent the primary economic input into livestock production systems. Metabolic and sensory factors affect short-term feeding behavior. Long-term feeding behavior is controlled by the animal in its attempt to achieve a defined equilibrium within its environment. Understanding mechanisms involved in regulating feeding behavior and appetite may lead to more efficient production of livestock and poultry.

Committed Goals: 1. Regulate intake to optimize use of feed resources. 2. Improve energy balance of neonatal livestock and poultry

Planned Approaches: 1. Elucidate roles of nutritional metabolites and hormones in regulating feed intake. 2. Identify hypothalamic factors that control systems regulating feed intake. 3. Identify physiological processes controlling neonatal feed intake and their interactions with stressors.

Expected Outcomes: 1. Improved efficiency of feed use by livestock and poultry. 2. Increased

neonatal survival rates.

Engaged ARS Locations: Athens, GA; Beltsville, MD; Clay Center, NE; Dubois, ID; East Lansing, MI.

Selected Accomplishments:

Pig liver cell culture system developed to determine the overall influences of regulatory hormones on hepatic metabolism (1). ARS scientists in collaboration with colleagues in Spain elucidated the roles of hormones on lipid oxidation, ketogenesis, ureagenesis, and glucose (glycogen) deposition. These cells were highly responsive to physiological levels of insulin, glucagon, thyroid hormone and glucocorticoids, but not leptin. The leptin receptor gene was identified and quantified in cultured liver cells; however, short-term treatment with leptin was not associated with alterations of any of the typical indicators of energy metabolism in these cells. The specific involvement of several of these hormones with hepatic energetics will provide valuable insight into whole body energy metabolism; provide data needed to formulate an overall strategy for regulation of growth in pigs; and clearly demonstrates that the liver is not a key target of the leptin-feed intake regulatory cascade. In addition, this cell culture methodology is now available and serves as a baseline for future studies on hepatic metabolism in pigs by other investigators.

Hormonal control of intracellular machinery in hepatocytes elucidated (2). Aquaporin 9 (AQP9) is a complex channel-forming protein that modulates water, glycerol and urea movement across the cell membrane, and thus plays a primary physical role in regulation of energy metabolism. An affinity-purified specific antisera to porcine AQP9 was prepared and characterized. Hormones that regulate energy utilization (glucagon, insulin and thyroid hormone) were all shown to impact the expression of AQP9 in hepatocytes. These results indicate that aquaporins are an integral component of the cellular response to a changing hormone environment. By comparing the new antisera with available human/rat antibody reagents these data demonstrate that it is imperative to use species-specific analytical tools to accurately investigate protein and gene expression in livestock animals. These data provide insight into how the hepatic cells regulate expression of intracellular machinery to rapidly exchange nutrients in response to hormonal stimulation. The fully characterized pig specific antisera is available as a research tool to other investigators.

Effects of feed deprivation on gene expression elucidated (3). Delay in the initiation of feeding can decrease post-hatch survival and adversely affect subsequent growth and body composition of broiler chickens. ARS scientists in conjunction with the University of Maryland investigated the effects of feed deprivation for 48 hr immediately following hatch on the expression of genes involved in lipid synthesis and the insulin-like growth factor (IGF) system (IGF-I, IGF-II, IGF-receptor, and IGF-binding proteins 2 and 5) in liver tissue from broiler chicks. Feed deprivation delayed, but did not diminish the dramatic surge in lipogenic enzyme gene expression observed between 24 and 72 hr post-hatch. Expression of the IGF system genes was also transiently affected by feed deprivation. These findings are important because they help to define some of the important early changes in gene expression that occur in response to the initiation of feeding behavior. This information is useful in assessing the consequences of feed deprivation in the neonatal chick that occurs naturally during the hatching process and during their subsequent transfer from hatcheries to grow-out facilities.

Uncoupling gene homologue identified and investigated in turkeys (4). Using molecular cloning technology, a unique uncoupling protein gene homologue was identified and sequenced in turkeys. In addition, expression of the uncoupling gene in broiler chickens was studied during periods of feeding and feed deprivation. This gene was found to be highly expressed in muscle tissue and may play a role in fat metabolism. These findings offer new insight into the control of energy expenditure in poultry that can have a direct impact on the level of lean versus fat tissue accretion in broiler chickens.

Problem Area VIB – Tissue Growth and Development

Problem Statement: Rapid and efficient growth is important for profitable animal production. Under market conditions with small profit margins for livestock and poultry producers, improved growth and efficiency is critical for economic survival of many producers. Optimum growth, performance, and efficiency have limited value if product quality is not acceptable to consumers. The impact of tissue development and growth on meat tenderness and composition is not understood. Knowledge of genetic factors and nutrition that control development and growth of muscle, fat, and mammary tissue is needed to develop practical methods for improving meat quality and composition and milk production.

Committed Goals: 1. Alleviate physiological and environmental conditions to enhance expression of growth potential by livestock and poultry. 2. Alter characteristics of meat products from livestock and poultry to improve palatability and nutritional value. 3. Determine the genetic and physiological basis for replication and differentiation of adipocyte precursor cells, muscle satellite cells, and mammary epithelial stem cells. 4. Identify tissue specific bioregulatory mechanisms for adipose, bone, muscle, and mammary tissue growth, and function.

Planned Approaches: 1. Investigate neural, endocrine, and immune mechanisms affecting growth and composition at animal and tissue levels. 2. Use whole-animal and in vitro models to understand and control developmental processes as they affect productivity and product quality. 3. Characterize growth- and immune- related endocrine function during tissue wasting that accompany parasitism and endotoxemia. 4. Manipulate differentiation of fat cells from fetal stromal vascular cells.

Expected Outcomes: 1. Increased growth rate and reduced length of production cycles in the livestock and poultry industries. 2. Livestock and poultry products with sensory attributes and nutrient composition desired by consumers. 3. Increased lactational persistency and efficiency of milk production in dairy cattle.

Engaged ARS Locations: Athens, GA; Beltsville, MD; Clay Center, NE; Dubois, ID; East Lansing, MI.

Selected Accomplishments:

Diabetic drugs identified to have impact on enhancing marbling deposition in swine (1). The National Pork Board has identified improving pork quality as a goal to increase pork demand. Consumer demands have led to greater focus on meat quality including tenderness, juiciness and flavor which are associated with intramuscular fat content. A novel technique was developed to allow simultaneous study of intramuscular or marbling fat cell development and subcutaneous fat cell development and was then used to study and screen agents that preferentially influence marbling fat cell development. Marbling fat cell development was induced by a group of orally active diabetic drugs called thiazolidinediones. A particular thiazolidinedione called troglitazone enhanced marbling fat deposition but did not influence carcass fat deposition or other growth traits. This discovery will lead to new treatments management systems which will significantly increase pork quality via alterations in fat tissue growth and development.

Leptin shown to reduce muscle wasting in swine during stress (2). Leptin treatment of rodents has been demonstrated to promote a negative energy balance, yet the tissue mobilization is specific to adipose, with a sparing of skeletal muscle, atypical for animals in negative energy balance. ARS scientists demonstrated that porcine leptin can alter protein accretion rates by muscle cells in vitro through reduction in protein breakdown. In addition, leptin was shown to increase fatty acid oxidation by muscle cells, suggesting the ability for leptin to partition specific nutrients for energy metabolism in

muscle. This work identifies the mechanism of action of leptin to reduce muscle wasting during periods of stress and subsequent muscle nutrient deprivation.

Uncoupling proteins offer promise in swine as pharmacological targets (3). ARS research examined whether genes associated specifically with heat production and energy loss, Uncoupling Proteins, are expressed in porcine adipose tissue and whether they are regulated by hormones. This study was the first to demonstrate that these uncoupling proteins are regulated by thyroid hormones and growth hormone in porcine adipose tissue. These data suggest that uncoupling proteins are pharmacological targets for altering heat loss and energy wasting in swine.

Unraveling of the biology of low-level inflammatory stress leads to identification of critical control points for improving animal growth (4). A subpopulation of beef animals was identified that displayed phenotypic characteristics of over-responsiveness and prolonged pathology response to low-level inflammatory stress. Termed “hyper-responders”, these animals constitute approximately 12% of the animals tested. These animals can be identified by the magnitude and duration of the clinical responses to repeated low-level endotoxin challenge and generally present a situation where unless very closely managed, survival past weaning is less than 50%. The ability to rapidly test for these animals may be facilitated by the discovery of a discrete nucleotide polymorphism in the TNF gene promoter region. The ability to select out and prevent the breeding of stock carrying genetic predispositions for increased disease susceptibility is drawing interest from beef producer groups in the U.S.

Research on the animal-to-animal variability in response to low-level immune challenge demonstrated that the oxidative conditions associated with low-level disease stress as modeled by endotoxin challenge, could efficiently cause nitration of specific regulatory sites of signal transduction kinases and negate the activation of the kinase, thus blocking initiation of downstream gene activities. Five specific critical control points were identified in the biochemical pathway between which the substrate for generation of nitric oxide (arginine) enters the cell, and the point at which vitamin E was shown to be effective in mitigating the generation of the reactive nitrogen product (peroxynitrite). Each critical control point constitutes a site at which potential beneficial intervention strategies could be targeted to reduce the severity of host response to immune challenge. The five points were: plasma membrane arginine transporter activity, arginase activity, xanthine oxidase activity, endothelial-type nitric oxide synthase, and protein kinase B/AKT. A key outcome of this research was that greater than 80% of the inflammatory compromise of metabolism could be prevented with the administration of vitamin E prior to the immune challenge. Two specific critical control points were identified in the biochemical pathway between the binding of growth hormone to its receptor and the inhibition of insulin-like growth factor-1 production as occurs during proinflammatory stress. This discovery points to a novel mechanism underlying metabolic perturbations that develop during inflammatory stress. The ability for vitamin E to modulate this observation suggests that non-pharmacologic intervention strategies could be used to lessen distress from inflammatory stress and lessen the use of antibiotics in cattle production. The impact of these observations is a better understanding of the biology of host response to combined stresses of weaning and vaccination and the development of specific measures that limit the production of free radicals and protein oxidation that lead to growth disturbances and economic loss in beef cattle management.

Analysis of mammary stem cells offers potential in dairy cattle (5). Previous ARS research characterized growth in the developing bovine mammary gland and identified the proliferative cell population of putative mammary stem cells, but little is known about regulation of the population size. ARS scientists, working with colleagues at Virginia Tech, completed studies to evaluate the impact of growth hormone and estrogen treatment (known stimulators of mammary growth) and ovariectomy (inhibitor of mammary growth) on the prevalence of mammary progenitor cells. The treatments induced significant changes in rates of proliferation with only small changes in the proliferating population, suggesting that small changes in the progenitor population may have profound effects on mammary growth and development. Current studies are utilizing additional methods for identification of stem cells,

which will then be isolated. The impact of treatments on gene expression within the putative stem cells will be studied in order to identify genetic pathways that regulate their growth and differentiation, and hence animal productivity. These studies have garnered significant scientific interest by researchers and industry representatives seeking to further these approaches to enhance mammary tissue growth.

Elevated energy intake prior to puberty retards mammary epithelial cell number (6). A series of ARS studies has elucidated information on mammary cell proliferation in the prepubertal dairy heifer: 1) Estrogen and its alpha receptor ($ER\alpha$) mediate the ovaries' influence on prepubertal mammary epithelial cell proliferation in cattle. However, the ER-beta isoform and the estrogen-related receptor $\alpha 1$ ($ERR\alpha 1$) may also play a role in estrogen-mediation of mammary growth or other biological functions. ARS research has shown that the $ER\alpha$ and $ERR\alpha$ were present during all physiological stages evaluated, suggesting a functional role for $ERR\alpha$ and a relative lack of role for $ER\beta$ in bovine mammary gland development and lactation. 2) In a collaborative study with investigators at Cornell University, it was demonstrated that reductions in mammary cell number at puberty were associated with increased energy intake. The decreased mammary cell number resulted from a reduction in time to puberty or constant body weight rather than impairment of epithelial cell proliferation rate. Among all heifers, there was a correlation between parenchymal $ER\alpha$ mRNA abundance and mammary epithelial proliferation rate. However, energy intake did not significantly influence parenchymal expression of these transcripts, nor did it affect mammary growth rate. Nutritional guidelines for rearing dairy heifers are strongly influenced by the potential impact of over-nutrition on mammary gland development. These data suggest that such concerns are over-emphasized. 3) In collaboration with investigators at the University of Illinois, a study was conducted to determine if long day photoperiod that hastens puberty could be used to promote lean body growth without limiting body stature or mammary growth in prepubertal heifers. Long day photoperiod hastened puberty and accelerated lean growth without limiting skeletal growth or mammary development of Holstein heifers. These and future studies will provide information regarding the regulation of mammary gland growth.

Milk production increases during early lactation due to an increase in secretory activity per cell rather than increases in the number of secretory cells (7). Previous research has suggested that increased thyroid hormone activation within the mammary gland and decreased activity in peripheral tissue serves to enhance partitioning of nutrients to the mammary gland of cattle. ARS research has now demonstrated that there is a rapid increase in the number of transcripts encoding the enzyme that activates thyroid hormones (thyroxine 5'-deiodinase, type II deiodinase) within the mammary gland during early lactation. Conversely, there is a decline in the number of transcripts for the enzyme that inactivates thyroid hormones (thyroxine 5-deiodinase, type III deiodinase). There is also an increase in transcripts for thyroid hormone receptor beta-1 in the mammary gland and a decline in transcripts encoding a nuclear receptor that interacts with, and alters the activity of, thyroid hormone receptors. These data support the concept that alterations in thyroid hormone metabolism and receptor activity increase mammary gland sensitivity to thyroid hormones during the transition from pregnancy to lactation. Greater understanding of factors that differentially regulate tissue sensitivity to thyroid hormones may enable manipulation of nutrient partitioning to further enhance efficiency of lactation.

Insulin-like growth gene expression studied in turkeys (8). There is currently very little information concerning which genes are responsible for regulating growth and development in the domestic turkey. ARS scientists have successfully cloned portions of the insulin-like growth factor-II (IGF-II) gene and studied its expression along with the expression of related genes in the IGF system (IGF-I, IGF-I receptor, IGF-binding proteins 2 and 5) in turkey liver and brain tissue obtained during embryonic and early post-hatch development. The IGF-I gene was expressed in liver only after hatching, whereas expression of IGF-II was expressed in both liver and brain tissue during embryonic development. This work provides new insight into the structure of the turkey IGF-II gene and suggests a role for the IGFs and their common receptor in the regulation of embryonic growth and development of the turkey.

RESEARCH COMPONENT VII: PRODUCT QUALITY

Livestock and poultry production provide consistently uniform and nutritious foods to the consumer. However, as consumer demands change and production systems evolve to meet those demands, high product quality must be maintained. As consumers demand lower fat foods, animal production systems respond with changes in breeding, nutrition, and management to decrease fat content. Consumers have many choices of food products, so competition among foods is very high. Factors create challenges to maintaining high sensory quality and satisfied consumers. New information is continually needed to provide food animal producers with the tools to continue to develop innovative products that meet processor and consumer needs.

Vision Statement: Livestock and poultry products of consistently superior quality.

Mission Statement: We seek to optimize product quality by controlling all sources of variation within geographic and economic constraints and by eliminating defective or substandard products from the marketplace.

Impact: Increased consumer satisfaction and demand for livestock and poultry products.

Linkages: USDA-ARS National Programs: 107 Human Nutrition; 108 Food Safety; 205 Rangeland, Pastures, and Forages; 207 Integrated Agricultural Systems; and 306 New Uses, Quality, and Marketability of Plant and Animal Products.

Other Agencies and Departments: USDA-CSREES, Montana State University, Prairie View A&M, Universities of Georgia, Idaho and Nebraska, Foreign Agriculture Service.

Private Sector: Arkansas Land and Farm Development Corp., Excel, Tyson, National, Swift, Future Beef.

Problem Area VIIA – Interactions of Genetics and Nutrition

Problem Statement: Altering dietary vitamin, mineral, energy, and protein levels affects product quality. However, incomplete understanding of mechanisms controlling product quality has resulted in variable responses to attempts to improve product quality through nutritional supplementation. Advances in genomic research provide new insight into mechanisms controlling phenotypic expression. In addition to facilitating efforts to genetically select for improved product quality, these advances increase the possibility of modifying product quality through nutritional supplementation. Some alleles may have antagonistic effects on production efficiency and product quality. Thus, a desirable goal may be to select for alleles that maximize production efficiency and offset any antagonistic effects on product quality through nutritional supplementation.

Committed Goals: 1. Optimize product quality through nutritional and metabolic modification of targeted biochemical pathways. 2. Nutritional regimes that optimize product quality in genetic strains that have been selected for improved production efficiency.

Planned Approaches: 1. Identify feeding/management strategies through altered dietary vitamin, mineral, energy, and/or protein levels to improve product quality. 2. Determine effects of novel and/or genetically-modified feeds on product quality. 3. Quantify interaction effects of animal genetics and nutrition on product quality.

Expected Outcome: 1. Enhanced sensory qualities and wholesomeness of livestock and poultry products.

Engaged ARS Locations: Beltsville, MD; Clay Center, NE; Dubois, ID; El Reno, OK; Miles City, MT.

Selected Accomplishments:

Selenium supplementation to enhance selenium in lamb meat products (1). Limited information is available describing the distribution of selenium in lambs consuming supranutritional selenium from organically bound sources. Scientists at the U.S. Sheep Experiment Station fed growing wether lambs a diet containing supranutritional selenium in the form of high selenium grain to determine the influence of time and dietary selenium concentration on selenium status and distribution. Most organs and tissues of the body were saturated with selenium early in the study, but skeletal muscle increased in selenium concentration throughout the duration of the study.

A stepwise process to rapidly and safely increase selenium depots in sheep and enrich lamb meat with selenium was developed. Specifically, lamb muscle can be enriched with selenium, when feeding naturally high-selenium wheat grain, at a predictable rate without adversely influencing lamb performance or health. Such a product could supply 100% of the human selenium RDA in as little as a 100-g serving, whereas a similar portion of average store-purchased lamb only supplies 15%. The National Research Council Committee charged with the 7th revision of the Nutrient Requirements of Sheep used this information to: 1) propose a greater maximum tolerable limit for selenium in sheep; 2) demonstrate that selenomethionine feed sources result in different whole-body selenium distribution patterns than do traditional selenium salts; and 3) demonstrate that selenomethionine feed sources may be used to create a selenium-enriched product for human consumption. This research provides information that will be used to develop strategic nutrient management programs to enhance whole body selenium status and increase the selenium content of lamb products.

Problem Area VII B – Biological Mechanisms Controlling Variation

Problem Statement: Sensory attributes are important drivers of demand for livestock and poultry products. Lack of understanding of biological factors regulating variation in product quality limits efforts to devise methods to ensure consistent high quality products. Often biochemical pathways or specific enzyme systems involved that affect certain product quality traits are known, but their regulation and control are not known.

Committed Goal: Methods to manipulate biological processes that will result in consistently high quality products.

Planned Approaches: 1. Determine regulatory steps in biological pathways controlling variation in specific quality traits. 2. Assess strategies to regulate biological pathways controlling variation in specific quality traits.

Expected Outcome: Enhanced product quality enabling production for specific markets.

Engaged ARS Locations: Athens, GA; Beltsville, MD; Clay Center, NE.

Specific Accomplishments:

Age-dependent changes in lamb tenderness (1). ARS research has determined that age-dependent changes in lamb tenderness are due to changes in sarcomere length and changes in the activity of

calpastatin and μ -calpain. Changes to the activity of the calpain system results in dramatic increase in the extent of postmortem degradation of myofibrillar proteins as animals mature from 2 to 8 months of age. This finding indicates that sheep production systems that encourage the harvesting of lambs at very young ages likely result in inferior tenderness of lamb products.

Calpain enzyme system involvement in postmortem proteolysis (2). ARS research has determined that over-expression of the calpastatin gene resulted in decreased postmortem proteolysis in skeletal muscle of mice. This finding provides direct evidence of the role of calpastatin and the calpastatin gene in regulating postmortem proteolysis and indicates that postmortem proteolysis in beef muscle could be enhanced by manipulation of the calpastatin gene. Postmortem proteolysis in skeletal muscle of conventional mice to those lacking (knockout) the p94 form of the calpain gene was evaluated and determined that the p94 gene does not affect postmortem proteolysis. This has provided convincing evidence that further study of the role of p94 in meat tenderness is not warranted and that research efforts should be focused elsewhere.

Mechanisms of the effects of various growth promotants and combinations of growth promotants on tenderness of five beef muscles (3). Under a trust agreement between ARS and Elanco Animal Health, ARS determined that there were only minor effects from any growth promotant on the calpain enzyme system activities in the longissimus muscle. However, some growth promotants reduced the amount of calpain tenderization during aging in all five muscles and reduced tenderness in two muscles. These data suggest that some growth promotants may increase muscle mass by reduced proteolytic capacity with subsequent reduced meat tenderness.

Sources of variation in tenderness of beef and pork muscles (4). Although the price of the high quality "middle meats" continues to increase, the price of the lower quality cuts that make up 60% of the carcass is declining, thus, reducing overall carcass values. ARS scientists characterized eleven major beef muscles and five major pork muscles for variation in tenderness, muscle shortening, connective tissue content, and postmortem protein degradation during aging. It was determined that there is a large amount of variation both within and among muscles for tenderness traits and tenderness related biochemical traits. These data have identified the sources of variation in tenderness in different muscles and will facilitate development of muscle specific interventions for improving the quality and value of lower quality beef cuts.

Problem Area VIIC – Predicting Product Quality or Defects

Problem Statement: Mismatched genetics and production practices leads to considerable variation in the quality of animal food products. Many defects in quality are only noticed by the consumer, when it is too late to take corrective action. Processors need non-invasive, non-destructive testing procedures to identify defects and measure product characteristics. Objective measures of determining value characteristics should allow processors to more effectively communicate differences to producers and give producers greater incentive to improve product quality.

Committed Goal: Objective automated systems to evaluate quality of livestock and poultry products.

Planned Approaches: 1. Assess the efficacy of state-of-the-art instrumentation to measure and/or predict product quality and to identify qualitative defects. 2. Develop integrated control systems for instrumentation to measure and/or predict product quality and to identify qualitative defects.

Expected Outcomes: 1. Effective communication of value between producers and processors. 2. Fewer defective products presented to consumers.

Engaged ARS Locations: Athens, GA; Beltsville, MD; Clay Center, NE; University Park, PA; and Wyndmoor, PA.

Selected Accomplishments:

MARC Beef Carcass Image Analysis System (1). ARS scientists developed a system to objectively evaluate the leanness of beef carcasses based on computerized image analysis. In comparison to existing systems for evaluation of carcass leanness, this system is expected to save the U.S. beef packing industry \$15 million dollars annually and allows for a more accurate assessment of the value of each individual carcass. After development and extensive testing, prototype versions of this system were transferred to the industry through a cooperative research and development agreement between ARS and IBP, Inc. (now known as Tyson Fresh Meats, Inc.), the world's largest beef packing company. ARS scientists, IBP, and an equipment vendor cooperatively modified this system for commercial use and were awarded two U.S. patents. Currently, major U.S. beef packing companies representing 50% of the fed-beef processing capacity are implementing this system. Use of this system will allow beef packers to objectively identify value differences between carcasses and compensate producers appropriately. This information will allow producers to better understand the impact of their management decisions on the value of their product. In turn, this information will allow for more efficient production of cattle that excel in leanness which will improve the profitability of beef production and the competitiveness of U.S. beef in the global marketplace.

Slice shear force process made available to the beef industry (2). ARS developed a highly-accurate method for tenderness classification of beef. One component of that system was a technique for measurement of meat tenderness which was termed slice shear force. Because of ease of measurement there has been growing interest in use of this technique for routine meat tenderness evaluation. Under a trust agreement between ARS and the National Cattlemen's Beef Association, ARS scientists trained six other institutions to conduct slice shear force as an objective measure of meat tenderness and then evaluated their abilities to use it. Repeatabilities among institutions for conducting slice shear force ranged from very high to moderately high. Use of slice shear force will increase the ability of institutions to accurately assess meat tenderness. This technology has allowed a major beef processor and a major retailer to develop and market a billion dollar premium branded beef product line.

Under another trust agreement between ARS and the National Cattlemen's Beef Association, ARS developed slice shear force procedures for 22 beef muscles including all of the major muscles and many minor muscles. Application of these procedures have greatly facilitated beef tenderness research. These procedures have allowed for larger scale studies that previously were not feasible while greatly reducing the cost of data collection.

Development, validation, and technology transfer of the MARC non-invasive tenderness prediction system (3). The most frequent source of consumer dissatisfaction with the eating quality of beef is inadequate tenderness. Previously, the only method to accurately predict whether or not a beef carcass would produce tender or tough steaks was to remove a steak from the carcass and evaluate tenderness mechanically, resulting in high cost due to product devaluation. Therefore, the beef industry has sought development of a non-invasive method for beef tenderness prediction. ARS meat scientists developed a non-invasive beef tenderness prediction system, incorporating the slice shear force technique, that was validated in several packing plants representing a broad sampling of cattle types and processing scenarios (including plants operated by three of the four major beef packing companies, smaller niche processors and branded beef companies). This research was conducted under a trust agreement between ARS and the National Cattlemen's Beef Association with cooperation and in-kind funding from five beef companies. ARS scientists collaborated with an equipment manufacturer to tailor the equipment for evaluation of beef carcasses. To date two companies are in the process of adopting this technology and others are in discussion with the equipment manufacturer. Based on the current

level of interest in adoption of this technology, it is expected to have an annual multi-million dollar impact and will benefit both consumers and all sectors of the beef industry.

Dual wavelength x-ray absorptiometry (DEXA) formalized as a means for predicting bone, lean and fat tissue (Richards 4). DEXA technology provides a rapid and noninvasive measurement of live body composition in swine and poultry. DEXA will revolutionize both the poultry and swine industries because parent stock of both animal species can be evaluated as breeder candidates by noting predicted stores of both body fat and protein and using these indices as part of a breeding strategy. Performance of offspring will be noted as functions of parent DEXA scores and will save the poultry and swine industries 25 million dollars annually because ineffective parent stock will be weeded out before their maintenance costs are amortized against costs of raising the progeny.

RESEARCH COMPONENT VIII: INTEGRATED SYSTEMS

Effective resource allocation is key to improving efficiency of production of livestock and poultry. Managerial and biological processes involved in the conversion of resources to a product by domestic animals are inherent components of a system. Traditional analytical approaches identify technologies to improve domestic animal production efficiency, but emphasize individual components of the system. Interactions among the components make typical domestic animal production systems complex and inconsistencies between experimental results and system performance may result. Integrating scientific knowledge using computer-based technologies facilitates improving production efficiency through understanding of entire production systems.

Vision Statement: Improved production efficiency through integration of knowledge.

Mission Statement: We seek to provide customers with decision aids which synthesize existing knowledge related to conversion of resources to end products and to environmental impacts of livestock and poultry production.

Impact: Objective and effective decision making by policy makers, production managers, consultants, and scientists in evaluating effects of policy, management alternatives, and in identifying needed research.

Linkages: USDA-ARS National Programs: 205 Rangeland, Pasture, and Forages; 206 Manure and Byproduct Utilization, 207 Integrated Agricultural Systems.

Other Agencies and Departments: USDA-CSREES, Universities.

Private Sector: National Cattlemen's Beef Association.

Problem Area VIIIA – User Information Packages

Problem Statement: Producers want access to knowledge allowing them to improve efficiency of producing livestock and poultry. Discipline-specific research has created a large body of information describing biological processes in components of production systems. Transfer of this information will be enhanced through use of computer models. Models that describe growth and production responses for crops and domestic animals, predict production environment characteristics, and whole farm models are available. These models were developed to address specific questions and are not structured for management decisions. Producers and other stakeholders may not have the resources necessary to apply these components to decision making. Decision support systems are one approach to overcome these limitations.

Committed Goals: **1.** Integrate information pertaining to crop growth environment, harvesting methods, storage conditions, and ration characteristics into feed evaluation systems. **2.** Evaluate whole farm management options, including feed production strategies, to reduce nitrogen in fertilizer and manure. **3.** Determine combinations of production inputs that optimize product quality within economic and biological constraints. **4.** Improved decision making by producers of livestock and poultry products.

Planned Approaches: **1.** Engage producers in identifying needed user information packages. **2.** Survey existing knowledge bases to obtain software modules that characterize biological process(es);

identify biological processes where existing modules are deficient or non-existent; and when necessary reformulate predictive functions. **3.** Use existing databases to test and evaluate new modules. **4.** Develop user-friendly information packages linking discipline oriented biological process modules into integrated components and structured models of production systems. **5.** Deliver information packages to producers for evaluation and acceptance

Expected Outcomes: **1.** User-friendly decision-support software that accurately predicts outcomes of biological processes involved in domestic animal production. **2.** More efficient use of resources in production systems. **3.** Reduced environmental impact from livestock and poultry production systems.

Engaged ARS Locations: Brooksville, FL; Clay Center, NE; Madison, WI.

Selected Accomplishments:

Decision Evaluator for the Cattle Industry (DECI) further validated and enhanced (1). ARS responded to the request from the National Cattlemen's Beef Association in the 1990s to develop a production systems support tool that would allow long-term evaluation of changes in cow-calf and feedlot production systems. The DECI model was the result of this work, first becoming available in 1997. DECI has been widely used by universities and the beef industry as a teaching and management decision tool. ARS researchers have validated it using a number of ranching systems in diverse geographical regions of the U.S. and abroad. The dynamic simulation model of a decision support tool for the beef industry that predicts beef animal performance requires updating to incorporate the latest understanding of biological processes. Several updates have been achieved, including: 1) A system for partitioning daily metabolizable energy intake was proposed, developed, incorporated into the simulation model and evaluated for accuracy. Results of accuracy evaluation document that revising the energy partitioning module enhanced the predictive performance of the simulation model. 2) A prototype version of DECI was developed expanding the capability of producers to evaluate cattle breeding programs. This version allows producers to characterize production potential of composite breeds of cattle. 3) Recent revision of the simulation model underlying DECI to predict nutrient partitioning provides beef cattle producers a tool to monitor the impact of their feeding strategies on the environment.

The National Beef Cattle Evaluation Consortium adopted DECI to evaluate risks of implementing genetic improvement programs at the commercial beef cattle producer level. This tool has been adopted both nationally and internationally by beef cattle managers to evaluate strategic management plans prior to implementation and is a very effective tool for transferring ARS animal production research to the industry.

Defined relationships between CP, NDF, and ADF that are the basis for national forage quality guidelines used by the USDA-Market News Service to report hay prices (2). In collaboration with the Market News Service, basic chemical analysis information was collected from commercial testing laboratories in 15 states. ARS US Dairy Forage Research Center researchers determined that relationships were similar among different regions of the U.S., indicating that a national standard was possible, but that relationships differed among laboratories. Least-squares and geometric regression was used to identify and define the relationships among CP, NDF and ADF that were consistent for the entire nation. These relationships were used to develop hay quality guidelines that are used by the USDA-Market News Service to report hay prices according to consistent forage quality standards throughout the U.S. This work was recognized in a Distinguished Support Award from the National Market News Association. The approach used is being incorporated in the dairy forage expert system.

Created a Corn Silage Fragmentation Index for quantifying the extent of kernel disruption in corn silage (3). Corn kernels in chopped whole-plant corn silage can often pass through dairy cows without being chewed or digested. This can result in a significant loss of digestible nutrients from silage,

especially if the corn silage is more mature and the kernels are dry and hard. Kernel processors (roller mills) have been added to silage choppers that complete the fragmentation of kernels during harvesting. However, numerous factors impact the effectiveness of kernel processing, such as width between the rollers, roller design and wear, and mass of material forced through the rollers per hour. ARS research documented that a sieving procedure could separate the whole kernels and large kernel fragments ($1/4^{\text{th}}$ of a kernel) in corn silage. The approach was combined with starch analysis to develop the corn silage fragmentation index, which is the percentage of starch that is in particles less than $1/4^{\text{th}}$ of a kernel that will be completely digested by cows. The index method provides producers a tool to reduce processing energy and maximize corn silage nutritive value. This index will be incorporated in the dairy forage expert system's evaluation of corn silage.

Developed an economical and simple system for measuring dry matter on farms (4). Precision feeding of dairy cows requires that the proper proportions of dry matter from every feed be fed each day. The dry matter of feeds often changes due to difference in lots of forage or to rain or snow contamination of feeds prior to feeding. Added moisture in feeds results in less forage being fed, which can cause health and performance problems for animals. Extension specialists conceived the idea of using a food dehydrator as an economical and easily available instrument that could dry feed samples unattended. They contacted ARS for assistance in developing a suitable method. Research was done to develop a method that was rapid, easy, precise and accurate. A suitable method was developed and presented to the Extension service and the public. This method will complement the output of the dairy forage expert system to improve precision feeding of animals.

National Program 105 – Animal Well-Being and Stress Control Systems Action Plan 2002-2007

The Agricultural Research Service (ARS), USDA, conducts research on high-priority problems facing U.S. and global food and fiber production. One of the significant mission areas is to conduct research to understand and optimize the care and well-being of production animals. Animal stress is both a societal and economic production concern. Improved states of well-being are known to be associated with better health, growth, and reproduction. Research programs within National Program 105 are partitioned into six components:

- **Scientific Measures of Well-Being and Stress**
- **Adaptation and Adaptedness**
- **Social Behavior and Spacing**
- **Cognition and Motivation**
- **Practices and Systems to Improve Care and Well-Being**
- **Bioenergetic Criteria for Environmental Management**

Vision: Measures of well-being and stress will be developed and refined, to give producers and consumers the information they need to evaluate management practices and determine which techniques best assure the care and well-being of animals used for food production.

Mission: The development of scientific measures of stress and well-being and an enhanced ability to interpret such measures is crucial to the evaluation of current agricultural practices and development of improved alternatives. The research strategy will focus on indicators of animal stress and well-being that can be refined and applied to the assessment of individual management practices and development of decision support systems. Stress caused by social, psychological, nutritional, and environmental stressors and the interactions of these stressors need to be understood to limit negative impacts on care, production efficiency and well-being. Animal stress and well-being research will benefit animals, producers, and ultimately consumers, by reducing animal health-care costs and improving food production efficiencies. Achievement of these economic and societal goals will help make U.S. animal products more affordable in domestic markets and competitive in world markets.

Problem: Food animal production is important to United States consumers as a major source of food nutrients and important to the national economy, being valued at about \$100 billion annually. Measures of animal well-being and stress are needed to give producers and consumers the information they need to evaluate management practices and determine which methods best assure the well-being and productivity of animals used for food production.

Approach: New knowledge is needed to understand well-being and stress. The new knowledge should come from research conducted in coordinated multidisciplinary studies integrating behavioral, physiological and productivity parameters and include evaluation of management methods in current and alternative systems to understand and manage well-being and stress. Research on transportation stress in relation to food safety will be conducted at two locations.

The program components, Adaptation and Adaptedness, Social Behavior and Spacing, and Cognition and Motivation involve specialized measures or experimental approaches involving behavioral

responses. These areas require emphasis because of the limited knowledge on farm animals.

Carrying-out a multidisciplinary integrated approach will contribute balanced scientific knowledge about each of the six components in this national program.

Committed Goal: To develop scientific measures of well-being and stress to give producers and consumers better information to judge which techniques best assure the well-being and productivity of animals used for food production.

Intermediate Outcome: Integrated measures of well-being and stress provide producers and consumers some of the information they need to evaluate management systems.

Long Term Outcome: Animal production systems that minimize stress and improve care to meet the well-being needs of animals and to address the concerns of consumers and producers.

Linkages: USDA-ARS National Programs: 101 Food Animal Production; 103 Animal Health; 108 Food Safety.

Other Agencies and Departments: USDA-CSREES, Mississippi State University, Purdue University, Texas Tech University, Universities of Missouri and Nebraska.

Engaged ARS Locations: Clay Center, NE; Columbia, MO; Lubbock, TX; Starkville, MS; West Lafayette, IN.

Problem Area IA: Scientific Measures of Well-Being and Stress

Measures of well-being of food producing animals are needed to make scientific assessments. These measures must be scientifically sound and relevant. The measurements will integrate behavioral, physiological, and productivity parameters of economic importance.

Selected Accomplishments:

Betaglucan and ascorbic acid enhances immune systems (1). Betaglucan plus ascorbic acid was fed to neonatal pigs and dairy calves in cooperative research to reduce weaning stress in piglets and/or transport stress in dairy calves. Both betaglucan and ascorbic acid (vitamin C) play individual roles through early response hormones and proteins in some tissues. This dietary supplement has been patented and will be useful to enhance well-being of dairy calves in transit and improved productivity of piglets kept in housing systems used by small "family" farms that are likely more stressful.

Development of multiple indicators (2). Many standard management practices in modern intensive production systems may subject livestock to suffering, but no reliable bank of indicators of stress and well-being are available. Acute and chronic stress-induced behavioral and physiological changes have been studied by ARS scientists and collaborators. Multiple indicators, including behavioral, neuronal, hormonal and immune parameters, have been developed. An index of these behavioral and physiological responses is being evaluated. These relationships should be useful to evaluate animal stress responses and well-being by producers to modify current practices and guidelines.

Tools developed to link stress and disease (3). Toll-like receptors identify pathogens and initiate the appropriate immune response, but little is known about their specific functions within cattle, swine and poultry. Primers and probes for molecular analysis of cattle and chicken receptors were developed to

distinguish among microbial pathogens (toll-like receptors). The effect of growth hormone and a stress hormone (glucocorticoid) on the expression of those receptors in dairy calves showed that expression of toll-like receptors changed over time. Lung tissue contained highly modulated receptors. During a lipopolysaccharide (a bacterial cell-wall component) challenge in chickens the toll-like receptor 2 is more responsive to the challenge than was toll-like receptor 4. Toll-like receptors were also highly modulated in lungs of chickens. This research developed the tools to explore the function of these important receptors during various stressors that farm animals experience and may elucidate the increased susceptibility of distressed animals to disease.

New Information on the fever response in baby pigs (4). Elucidating the immunological and physiological response of the young pig to a live *E. coli* bacterial challenge is important to understand the mechanisms associated with the febrile response in the young pig. Results from live bacterial challenges in young pigs questions the validity of existing dogma that tumor necrosis factor-alpha, a primary mediator of the acute phase immune response, is a primary mediator of fever in the young pig. This discovery could aid in developing novel approaches to treating bacterial outbreaks in swine.

Pregnant sows' stress carried-over to offspring (5). Research was conducted that examined the effect that stressing a pregnant sow has on her resulting offspring. It was found that alterations of maternal cortisol concentration increased the concentrations of corticosteroid-binding globulin (a stress protein) in 4-mo-old pigs that came from dams that were stressed during gestation when they were subjected to mixing. No consistent alterations were found in plasma cortisol in contrast to data in rodents. These data, as well as previous data from this research project, indicate that prenatal stress can alter the physiology of swine. Environmental influences on farm animals during gestation warrant additional critical investigations to understand the impact on production.

Problem Area IB: Adaptation and Adaptedness

Most food animals have been domesticated for thousands of years. Selection under intensive management conditions has occurred only recently and is oriented primarily toward the improvement of production traits. Research in this area will determine the roles that genetics and environment play in well-being. Research information on adaptedness will serve as the basis for modifying management practices. Genetic research will be evaluated to improve animal fitness and determine the basis of adaptation to environmental stressors such as heat and cold. Marker assisted selection techniques will be explored. (Some research that could be in this component is conducted in NP 101.)

Selected Accomplishments:

Genetically selected chickens more suited to production systems (1). Research demonstrated that animal well-being can be improved when selection is on production traits and competitive interactions are taken into account, by which antisocial behaviors, such as cannibalism and aggression, are overcome. The new selection program can be adopted by scientists and the breeder industry in developing new chicken strains with greater adaptation to the production system, with an emphasis on improving animal well-being and maintaining economic efficiency. This approach was shown to poultry breeders such as Hy-Line International. Research has shown that dopamine and serotonin, and immunity are useful indicators of stress and animal well-being of chickens for resistance to stress in modern production systems. Genetic selection plans involving behavioral traits have promise for improving the fitness of food animals for modern production systems.

Problem Area IC: Social Behavior and Spacing

With the intensification of animal agriculture and the greater number of animals at each location or in production units, a major question is whether intensive management adversely affects an animal's well-

being. Research will be conducted to provide a scientific basis for understanding the social behavior of food animals and how the quality and quantity of space influences behavior. Research to show consequences, such as changes in patterns of social interaction and space utilization, will require an integrated research approach.

Selected Accomplishments:

Early age imprinting to solve uneven distribution in housing (1). The effect of early environmental enrichment was determined on behavioral and physiological development in chicks. Visual imprinting during early life promotes brain structure development and improves spatial memory in chicks. The method could be adapted by producers to improve the well-being of chickens and reduce mortality resulting from physical and social stress in a large commercial-housing environment by reducing the problems associated with poor navigation and uneven distribution on the floor.

Optimal stocking density varies with final broiler market weight (2). In the United States, day-old chicks are stocked in poultry houses at densities of 13-17 birds/m sq. However, recent interest by the poultry industry is on stocking density at placement in chicken houses of day-old broiler chicks as a function of projected market weight (varies from 3.7 to over 9 pounds), with emphasis on "bottom line" efficiency. Research demonstrated stocking densities of 25-40 kg/body weight/m² for 3 kg chickens exhibit no reduction in growth rate. At a stocking density of 40-45 kg/body weight/m², reduction in growth rate occurs and 95% of the reduction is attributable to reduced feed consumption. Further, stocking densities of 25-45 kg/body weight/m² do not detrimentally impact stress hormone concentrations. This research will aid broiler producer's decisions on stocking density rates for maximal profitability.

Sow management systems evaluated (3). Gilts in mid-gestation were used by scientists to determine the effects of two penning systems (crates vs. pens of five) and feeding system (drop fed vs. trickle fed) on gilt behavior, pathogen shedding, immune measures, and production of breeding and gestation effects. Neutrophil phagocytosis efficiency may be improved for crated gilts that were trickle rather than drop fed. Among penned gilts, trickle feeding reduced neutrophil efficiency compared with neutrophils from gilts that were drop fed. Overall behavioral activity levels were statistically similar among treatments. Pregnant gilts expressed different forms of activity depending on the available space. Overall productivity of breeding and gestating gilts was similar in the four systems evaluated. There were no significant differences in pathogen shedding among trickle fed sows.

Problem Area ID: Cognition and Motivation

The mental state, fear, frustration, suffering, pleasure, and boredom of animals are major concerns of the public, however; there is currently little scientific information that can be used as a basis for addressing these concerns. Research is needed to learn how sensory information from the environment is perceived and processed by animals and what animals learn.

Selected Accomplishments:

The cognition part of this component is a more fundamental aspect of behavior that did not result in finished products during the time covered by this report. Motivation activities are parts of research reported under other components, such as the molting work without hunger.

Problem Area IE: Practices and Systems to Improve Care and Well-Being

Management practices, such as transportation and slaughter, and special agricultural practices, such as beak trimming, dehorning, branding, tail docking, and castration, are important and necessary elements

of animal management in current production systems. These practices affect the well-being of animals. Research will address evaluation of the current and alternative practices concerning potential pain, stress or discomfort, and production efficiency. Alternative environment systems and current management practices will be evaluated for their effect on farm animal well-being and overall goals to improve animal comfort, well-being, and production efficiency. Research to improve both production efficiency and animal well-being will be conducted.

Selected Accomplishments:

Molting without feed withdrawal (1). Incorporating melengestrol acetate (MGA) into a balanced layer diet induces molting in hens while they are maintained on a balanced layer diet. Maintaining hens on a balanced layer diet throughout molting prevented hen weight loss and decreased a hen's motivation to obtain feed. Inducing molt by MGA, allowed for a quick recovery to peak egg production and increased internal and external egg quality. This research is one of the first to show that hens can be 'molted' (reproductively rejuvenated) without causing hunger.

An alternative to gestation stalls (2). Use of sow gestation stalls is an animal well-being issue of national scope. Research was conducted that evaluated relevant indicators of behavioral and physiological stress for stall and group gestation housing of swine. Small group housing was a suitable alternative to gestation crates for gilts, and resulted in piglets with greater weight gain. This study demonstrated a potentially viable alternative of small alterations of present housing to allow more movement and social contact for gestating gilts.

A single neonatal treatment reduces age-to-market 5 days (3). Early hormonal therapy can have lasting effects on pig growth and performance. Additional research continued to evaluate the lasting effects of glucocorticoid therapy in neonatal pigs shortly after birth. Research conducted on a commercial swine farm in Missouri demonstrated that pig growth from birth to market age could be enhanced with a single injection of dexamethasone injection within 24 hours of birth. This research provides an approach for swine producers to shorten the time from birth to market by approximately 5 days, thus potentially saving millions of dollars annually.

Height of water nipples critical (4). Chickens drink almost twice as much water compared to feed consumption. Therefore, nipple waterer height was investigated relative to chick height. Research was conducted that showed nipple waterer placement must be based on the height at eye level of the shortest chick. Research showed approximately 6% mortality in chicks where waterer nipples are as little as 1½ inches higher than the height suggested by the manufacturer. Attention to this seemingly minor management detail can greatly improve profitability of the broiler industry. The nipple watering system was automated in relation to broiler growth to save labor.

Transport stress defined by age for calves (5). Transportation of cattle is an unavoidable stressor and dairy cattle are often transported within the first week of life, but we do not know how this affects their immune development and growth. To better find physiological and behavioral indicators of cattle not coping with that stressor, transport stress in cattle is being investigated. Proteins produced by the liver (acute phase proteins) were determined to be useful indicators of well-being for weaned calves and neutrophil functions and general health assessments indicated that transport at 2 to 3 days-of-age may be increasing susceptibility to respiratory pathogens and transport at 4 to 5 days-of-age increases susceptibility to enteric pathogens compared to calves transported at 6 to 8 days-of age. These studies showed that acute phase proteins are a useful assessment of individual mature cattle's ability to cope following stress and the second study indicated that methods are needed to improve the coping capability of young calves following transport at less than 1 wk-of-age.

Enhancing immune function with nutritional supplements (6). Research was continued to explore

the use of nutritional supplements to enhance immune function in weaned pigs as a means of reducing sub-therapeutic levels of antibiotics in swine feed. Research was conducted to evaluate the use of spray dried plasma and fish oil supplementation to enhance immune function in weaned pigs. This research expands scientific knowledge on nutritional regulation of the immune system. This research is stimulating interest in the use of nutritional alternatives to the use of sub-therapeutic levels of antibiotics.

Hormone found to enhance feed intake (7). Reduced feed intake at the time of weaning is a significant problem in swine production and has implications in overall performance, health, and well-being. Research was conducted on the newly discovered appetite regulation hormone (ghrelin) to enhance feed intake in newly weaned pigs. The endocrine profiles of young pigs were shown undergoing a period of voluntary feed restriction. The studies demonstrated that voluntary feed restriction at weaning may be prevented by providing exogenous ghrelin to the newly weaned pig. Implementation of these findings could significantly improve swine health and performance with ensuing economic impact.

Higher air velocity improves performance of broilers over 28 days of age (8). Since the mid to late 1980's, the majority of chicken houses in the southeast have utilized tunnel ventilation to keep chickens cool; however, the optimal air velocities for various ages has not been determined. Scientists evaluated air moving at 180m/min (600 ft/min) or 120m/min (400 ft/min) as compared to still air [15m/min (50 ft/min)] on body weight gain (BWG) and feed consumed relative to weight gain (feed:gain) of chickens. No improvement in the BWG or feed:gain was seen for increased air velocity as compared to still air for chickens from 21 days of age to 28 days of age; however, improvements were noted in both BWG and feed:gain for the increased air velocity for birds over 28 days of age. Even more dramatic was an improvement in both BWG and feed:gain from 42 to 49 days of age for air velocity of 600 ft/min as compared to the 400 ft/min. This research re-defines the optimal rearing and managerial conditions for growth of broilers by providing another factor for reducing the cost of poultry production.

Enriched cages compared to battery cages (9). Hens housed in enriched cages compared to those housed in battery cages have a greater bone mineral density and display more comfort behaviors, such as nesting and dust-bathing. Research demonstrated that moderate increase of flock density did not result in reduction in production. However, the density should be kept low because the higher stocking density could cause more stress for hens even housed in an enriched environment. This research is coordinated with a poultry cage manufacturer and shows that cage designs should be based on the genetic characteristics of the flocks. The results have been presented at various scientific meetings and two publications are being prepared.

Treating baby pigs with antibiotics may be detrimental (10). Treating an outbreak of *E. coli* in nursery pigs with systemic bactericidal antibiotics is a routine procedure in commercial swine production. Scientists demonstrated that the acute-phase response in pigs experimentally infected with *E. coli* and treated with systemic bactericidal antibiotics was more pronounced than in pigs infected with *E. coli* and not treated with systemic bactericidal antibiotics. These data suggest that treating *E. coli*-infected nursery pigs with systemic bactericidal antibiotics may be more detrimental to the pig than allowing them to recover on their own. This information will be critical to swine veterinarians for treating *E. coli* outbreaks in commercial herds and may allow new protocols to be developed to reduce overall mortality in nursery pigs.

New multivariable management tool (11). Scientists developed a mixed experimental design management tool for optimizing feeding of poultry. The design allows for optimization of multiple variables such as, body weight, feed conversion, carcass weight, breast yield, etc., through the provision of diets for specific and individual flocks. This study was accomplished utilizing ten treatments (various times of providing NRC-based starter, grower and finisher diets) for a total of 48 days of production in which the diets changed at specific times. The outcome is a new approach that permits poultry

producers to optimize the provision of poultry diets to minimize costs and maximize meat yield for a 48-day production period. This tool will have other variables added to evaluate animal well-being and environmental issues.

Problem Area IF: Bioenergetic Criteria for Environmental Measurement

Adverse environmental conditions cause livestock and poultry losses, decreased production efficiency, and decreased animal well-being. Available technology needs to be adapted for proactively managing environmental stressors. Research to develop decision support tools is needed to help producers deal with environmental stressors, provide protective measures, recognize livestock and poultry in distress, and take appropriate management actions.

Selected Accomplishments:

Identified early heat-stress indicator (1). Through comparisons of various responses of cattle to laboratory controlled heat stress conditions, ARS scientists have found and reported that respiration rate is an excellent indicator of stress in cattle for several reasons. First, respiration rate leads other forms of measurable response by several hours. Core body temperature increase is often considered a primary indicator of the level of stress, however, animals increase their rate of breathing before body temperature increases, probably in an effort to increase heat dissipation. Likewise, heat production increases as body temperature increases, but respiration rate precedes this increase as well. Behavioral changes can also signal increased stress levels, but behavioral changes can be subtle and not always immediately observable. Respiration rate is also more easily measured than heat production or core body temperature by observation of flank movements without disturbing animals. These visual indicators of stress are vital to cattle producers as a means to recognize the impact of the stress that is not solely dependent on a single climate measure. Heat waves that negatively affect cattle are a combination of weather conditions, including environmental temperature, solar radiation, humidity, and air movement. These primary climatic measurements were combined into a simple mathematical relationship to predict the respiration rate. The mathematical relationship includes calibrations for coat color, body condition score, and respiratory health history. Thresholds of respiration rates were identified and quantified for the response to be in normal, alert, danger, and emergency categories. “Real time” local weather variables were incorporated to provide cattle producers with these predicted indicators and levels of expected cattle responses so that producers are aware of critical weather conditions and probable animal stress, thus able to take action to ameliorate the effects to the best of their ability by implementing planned management responses. This technology is in a prototype instrument being evaluated at several feedlots and will provide producers early warning of severe weather events.

APPENDIX 1 - SELECTED SUPPORTING INFORMATION AND DOCUMENTATION FOR ACCOMPLISHMENTS AND IMPACT OF NP 101 AND 105 RESEARCH

Planning and Coordination for NPs 101 and 105 National Workshops

- NP 105 Stakeholder Workshop, 1999
- Food Animal Integrated Research (FAIR 2002) Workshop, 1999
- NP 101 Stakeholder Workshop, 2000
- Joint USDA ARS-CSREES Animal Agriculture Stakeholders Workshop, 2001
- National Animal Germplasm Program Policy and Coordinating, Annual, 1999-2005
- Future Trends in Animal Agriculture Annual Symposia 2002-2005
- ARS Poultry Production Workshop, 2003
- Chicken Genome Workshops, Annual 2002-2005
- DISCOVER Conference on Antibiotic Use in Animal Agriculture, 2003
- ARS Swine Production Workshop, 2003
- Bovine Genome Workshops, Annual 2003-2004
- USDA Animal Genomics Workshop, 2004
- DISCOVER Conference on Animal Germplasm, 2004
- CSREES Animal Stress (W-173) and Animal Care/Behavior (NCR-131) Coordinating Committees, Annual, 2002-2005

NATIONAL PROGRAM 101 – FOOD ANIMAL PRODUCTION

Component I: Reproductive Efficiency

Problem Area Ia: Environmental Effects

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Problem Area Ic: Gamete and Embryo Storage, Sexing, Cryopreservation, and Use

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Problem Area IIb: Preserving Genetic Resources

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Patent: ARS researchers in the Biotechnology and Germplasm Laboratory at Beltsville, MD developed and tested an integrated group of technologies that would enable consistent cryopreservation and subsequent post-thaw viability of swine embryos. This suite of technologies, collectively referred to as the 'USDA Swine Embryo Cryopreservation Technology', was patented in FY 2003 (US Pat. No. 6,503,698).

Problem Area IIc: Information Systems

1. The primary product of the information systems component is the Genetic Resources Information Network (GRIN) database and user interface. The interface address is <http://sun.ars-grin.gov:8080/j2ee/nagppub/jsp/nagp/index.jsp>.

Component III: Genetic Improvement

Problem Area IIIa: Develop Breeding Objectives

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Problem Area IIIb: Accelerate Selection Response

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Marker genotypes and analysis information for the *DGAT1* and *FEZL* QTN were provided to collaborators (e.g. dairy AI studs, academic collaborators) in the Cooperative Dairy DNA Repository (CDDR) and to a multi-national pharmaceutical company (Merial). This information is being used to evaluate the commercial effectiveness of gene-based MAS programs based on marker tests potentially available to U.S. producers.

Semen for DNA extraction was distributed to collaborators at Monsanto, Semex Inc. (Canada) the Universities of Guelph, Wisconsin and Missouri and North Carolina State University to use as a resource for QTL investigations.

As a part of an SCA with USDA, ARS, MARC (Clay Center, NE) and Genaissance Pharmaceuticals (New Haven, CT), DNA from 96 of the most influential bulls of the major North American dairy breeds was assembled as a resource for determining allele frequencies of a set of markers being developed as national standards for parentage identification and animal traceback studies. To date, 30 SNP markers with appropriate major and minor allele frequencies in all breeds have been identified and made publicly available.

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Problem Area IIIId – Transgenic Livestock and Poultry

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Component IV: Genomic Tools

Problem Area IVa: Comprehensive Maps

1. Wallis, J.W., Aerts, J., Groenen, M.A., Crooijmans, R.P., Layman, D., Graves, T.A., Scheer, D.E., Kremitzki, C., Fedele, M.J., Mudd, N.K., Cardenas, M., Higginbotham, J., Carter, J., McGrane, R., Gaige, T., Mead, K., Walker, J., Albracht, D., Davito, J., Yang, S.P., Leong, S., Chinwalla, A., Sekhon, M., Wylie, K., Dodgson, J., Romanov, M.N., Cheng, H., de Jong, P.J., Osoegawa, K., Nefedov, M., Zhang, H., McPherson, J.D., Krzywinski, M., Schein, J., Hillier, L., Mardis, E.R., Wilson, R.K., and Warren, W.C. 2004. A physical map of the chicken genome. *Nature* 432:761-764.
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7. Swine BAC map information is available at the Sanger web-site (http://www.sanger.ac.uk/Projects/S_scrofa/mapping.shtml).

The MARC swine genetic linkage map is available at the MARC web-site (<http://www.marc.usda.gov/>) and the information is also mirrored at the National Center for Biotechnology information (NCBI; <http://www.ncbi.nih.gov/>), Iowa State University's web-site (<http://www.animalgenome.org/pig>) as well as at the PiGMAP web-site located at the Roslin Institute in the UK (<http://www.thearkdb.org/anubis?singleSpecies=pig>).
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Problem Area IVb: Genotyping Systems

1. Proceedings of the Bovine Genome Workshop. 2005. Houston, Texas.
<http://www.csrees.usda.gov/nea/animals/pdfs/bovine/agendaforweb.pdf>

Problem Area IVc: Tools and Reagents

1. Niikura, M., Dodgson, J., Cheng, H. 2004. Characterization and complete sequence of an infectious Marek's disease virus bacterial artificial chromosome clone generated from a virulent strain. *International Marek's Disease Symposium Abstracts and Proceedings*. P. 17.

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Problem Area IVd: Genomic Enhancement Systems

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Problem Area IVe: Bioinformatics and Statistical Tools

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Connor, E.E., Sonstegard, T.S., Keele, J.W., Bennett, G.L., Williams, J.L., Papworth, R., Van Tassell, C.P., Ashwell, M.S. 2004. Physical and linkage mapping of mammary-derived expressed sequence tags in cattle. *Genomics* 83:148-152.

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A software package (GenoProb) that extracts segregation information from genetic marker data in complex pedigrees with marker data on only a fraction of the individuals was developed. GenoProb has been made publicly available.

3. Matukumalli, L.K., Grefenstette, J.J., Sonstegard, T.S., Van Tassell, C.P. 2004. EST-page - a simple web interface for managing and analyzing EST data. Bioinformatics, 20:286-288.

The following are a selected sampling of the types of work facilitated by the bioinformatics infrastructure developed at BARC:

Sonstegard, T.S., Capuco, A.V., White, J., Van Tassell, C.P., Connor E.E., Cho, J., Sultana, R., Shade, L., Wray, J.E., Wells, K.D., and Quackenbush, J. 2002. Analysis of bovine mammary gland EST and functional annotation of the Bos taurus gene index. Mammalian Genome 13(7):373-3.

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Zhu, J.J., Lillehoj, H.S., Allen, P.C., Van Tassell, C.P., Sonstegard, T.S., Cheng, H.H., Pollock, D., Sadjadi, M., Min, W., Emara, M.G. 2003. Mapping quantitative trait locus associated with resistance to avian coccidiosis and growth. Poultry Science 82:9-16.

Ashwell, M.S., Heyen, D.W., Sonstegard, T.S., Van Tassell, C.P., Da, Y., Van Raden, P.M., Ron, M., Weller, J.I., Lewin, H.A. 2004. Detection of quantitative trait loci affecting milk production, health, and reproductive traits in Holstein cattle. Journal of Dairy Science. 87(2):468-75.

Blomberg, L., Long, E.L., Sonstegard, T.S., Van Tassell, C.P., Dobrinsky, J.R., Zuelke, K.A. 2004. Serial analysis of gene expression (sage) during elongation of peri-implantation porcine trophectoderm (conceptus). Physiological Genomics 20:188-194.

Van Tassell, C.P., Sonstegard, T.S., Ashwell, M.S. 2004. Mapping quantitative trait loci for dairy form in regions of chromosome 27 in two families of Holstein. Journal of Dairy Science 87(2):450-457.

Baumann, R.G., Baldwin, R.L., Van Tassell, C.P., Matukumalli, L.K., Sonstegard, T.S. 2005. Characterization of a normalized cDNA library from bovine intestinal muscle and epithelial tissues. Animal Biotechnology 16(1):17-30.

Schnabel, R.D., Jong-Joo, K., Ashwell, M.S., Sonstegard, T.S., Van Tassell, C.P., Connor, E.E., Taylor, J.F. 2005. Fine-mapping milk production quantitative trait loci on bta6: analysis of the bovine osteopontin gene. Proceedings of the National Academy of Sciences 102:6896-6901.

Over 60,000 EST have been processed and submitted through EST-PAGE at BARC alone. At least 28 other research groups in 10 countries, including France (3 sites in INRA and CNRS), England, Norway, The Netherlands, and Taiwan (4 sites) and the U.S. have requested and obtained EST-PAGE since it was released.

Component V: Nutrient Intake and Use

Problem Area Va: Regulating Gene Function

1. Swanson, K., Freetly, H.C., Ferrell, C.L. 2004. Nitrogen balance in lambs fed low quality brome hay and infused with differing proportions of casein in the rumen and abomasum. *Journal of Animal Science*. 82:502-507.

Bell, A.W., Ferrell, C.L., and Freetly, H.C. 2005. Pregnancy and fetal metabolism. In: Dijkstra, J., editor. *Quantitative Aspects of Ruminant Digestion and Metabolism*. 2nd edition. CAB International, Wallingford.
2. Swanson, K., Freetly, H.C., Ferrell, C.L. 2004. Nitrogen balance in lambs fed low quality brome hay and infused with differing proportions of casein in the rumen and abomasum. *Journal of Animal Science*. 82:502-507.

Swanson, K., Freetly, H.C., Ferrell, C.L. 2004. Nitrogen balance in lambs fed a high-concentrate diet and infused with differing proportions of casein in the rumen and abomasum. *Journal of Animal Science* 82:495-501.
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4. Ferrell, C.L., Berry, E.D., Freetly, H.C., and Miller, D.N. Influence of genotype and diet on steer manure odor, and carriage of pathogenic and other fecal bacteria. I. Animal performance. *Journal of Animal Science*. (Submitted 10-05)
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7. The following gene sequence data was submitted to the NCBI GenBank database and made available to scientists worldwide: Proszkowiec-Weglarz, M., Richards, M.P., Poch, S.M. 2005. *Gallus gallus* 5'-AMP-activated protein kinase gamma-1 non-catalytic subunit (PRKAG1), transcript variant 2, mRNA, complete cds. GenBank Accession Number DQ133598.

Proszkowiec-Weglarz, M., Richards, M.P. 2005. Identification and expression of the AMP-activated protein kinase pathway in chickens. *Poultry Science* 84 (Supplement 1):56 (Manuscript in press).
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Problem Area Vb: Interactions Affecting Reproduction

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Barb C.R., Hausman G.J., and Czaja K. 2005. Leptin: A metabolic signal affecting central regulation of reproduction in the pig. *Domestic Animal Endocrinology* 29:186-192.
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Taylor, J. B., J. W. Finley, and J. S. Caton. 2005. Effect of the chemical form of supranutritional selenium on selenium load and selenoprotein activities in virgin, pregnant, and lactating rats. *Journal of Animal Science* 83:422-429.
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Problem Area Vc: Microbial Effects

1. Faciola, A.P., Broderick, G.A., Hristov, A.N., and Leao, M.I. 2005. Effect of different levels of lauric acid on ruminal protozoa, fermentation pattern, and milk production in dairy cows. *Journal of Dairy Science* 88 (Suppl. 1): 178.
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Fox, D.G., T.P. Tylutki, L. O. Tedeschi, M.E. Van Amburgh, L. E. Chase, A. N. Pell, T. R. Overton, and J. B. Russell. 2003. The Net Carbohydrate and Protein System for Evaluating Herd Nutrition and Nutrient Excretion (CNCPS version 5.0). Cornell University, Animal Science Department Mimeo 213, Ithaca NY.

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Problem Area Vd: Minimizing Production Losses

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Aiken, G.E., Tabler, S.F., Looper, M.L. Brauer, D.K., Strickland, J.R. and Schrick, F.N. 2005. Influence of stocking rate and implantations on growth performance and rate of recovery from heat stress for steers grazing endophyte-infected Kentucky-31 tall fescue. *Journal of Animal Science* (Submitted for review).

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Problem Area Ve: Nutrient Use and Feed Evaluation

1. Marita, J.M., Ralph, J., Hatfield, R.D., Guo, D., Chen, F., Dixon, R.A. Structural and compositional modifications in lignin of transgenic alfalfa downregulated in caffeic acid O-methyltransferase (COMT) and caffeoyl-coenzyme A O-methyltransferase (CCOMT). Phytochemistry. 2003. v. 62. p. 53-65.
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Component VI: Growth and Development

Problem Area VIa: Regulating Feed Intake

1. Fernández-Fígares, I., Shannon, A.E, Wray-Cahen, D., Caperna, T.J. 2004. The role of insulin, glucagons, dexamethasone and leptin in the regulation ketogenesis and glycogen storage in primary cultures of porcine hepatocytes prepared from 60 kg pigs. *Domestic Animal Endocrinology* 27:125-140.

A CRADA was established and funded by a biotechnology company to investigate a new subclone of the ARS-PICM-19 porcine liver stem cell line that has unique hepatocyte-like morphology and function. These cells will be used to elucidate the regulation of gene expression and nutrient metabolism, and will provide valuable information as to the usefulness of this cell line for the construction of a bioartificial liver for use in human

medical applications. A website (www.hepalife.com) was developed which focuses on liver biology, liver pathology and on the ARS-PICM-19 cell line. The potential success of this cell line toward human medical applications is unlikely to be available to the medical community for 10 years. The primary constraint is the need for greater knowledge of the molecular genetics of hepatic immunity expression, hepatic architecture and tissue integration.

2. Talbot, N.C., T.J. Caperna, and W.M. Garrett. 2003. Analysis of the expression of aquaporin-1 and aquaporin-9 in pig liver tissue: Comparison with rat liver tissue. *Cell Tissues Organs* 174:117-28.
3. Richards M.P., Poch S.M., Coon, C.N., Rosebrough, R.W., Ahswell, C.M., McMurtry J.P. 2003. Feed restriction significantly alters lipogenic gene expression in broiler breeder chickens. *Journal of Nutrition* 133:707-715.
4. Evock-Clover, C.M., Poch, S.M., Richards, M.P., Ashwell, C.M., McMurtry, J.P. 2002. Expression of an uncoupling protein gene homolog in chickens. *Comparative Biochemistry and Physiology Part A*. 133:345-358.

Problem Area VIb: Tissue Growth and Development

1. Poulos, S.P, Hausman, G.J., Azain, M.J. U.S. Patent Application Docket No. 0178.03; entitled "Use of Thiazolidinediones to Increase Intramuscular Fat in Livestock."
 2. Ramsay, T.G. 2003. Porcine leptin inhibits protein breakdown and stimulates fatty acid oxidation in C2C21 myotubes. *Journal of Animal Science* 81:3046-3051.
 3. Ramsay, T.G., Rosebrough, R.W. 2005. Regulation of uncoupling proteins 2 and 3 in porcine adipose tissue. *Domestic Animal Endocrinology*. 28:351-366.
 4. Kahl, S., Elsasser, T.H. 2004. Endotoxin challenge increases xanthine oxidase activity in cattle: effect of growth hormone and vitamin E treatment. *Domestic Animal Endocrinology* 26:315-328.
- Elsasser, T.H., Kahl, S., MacLeod, C., Nicholson, B., Sartin, J.L., Li, C. 2004. Mechanisms underlying growth hormone effects in augmenting nitric oxide production and protein tyrosine nitration during endotoxin challenge. *Endocrinology* 145:3413-3423.
- Elsasser, T.H., Blum, J.W., Kahl S. 2005. Characterization of calves exhibiting a novel inheritable Tumor Necrosis Factor- α hyper-responsiveness to endotoxin: associations with increased pathophysiological complications. *Journal of Applied Physiology* 98: 2045-2055.
- Daniel, J.A., Elsasser, T.H., Martinez, A., Steele, B., Whitlock, B.K., Sartin, J.L. 2005. Interleukin-1 β and tumor necrosis factor- α mediation of endotoxin action on growth hormone. *American Journal of Physiology* 289:E650-657.
- Patent Application: T. H. Elsasser, S. Kahl, E. Connor and C. Ashwell. 2004. Patent application characterizing the biochemical nature and identification of the tumor necrosis factor- α hyperresponder animals and associated nucleotide polymorphisms in the TNF promoter region of the gene was submitted to the Office of Technology Transfer.
5. Berry, S.D.K., Jobst, P.M., Ellis, S., Howard, R.D., Capuco, A.V., Akers, R.M. 2003. Mammary epithelial proliferation and estrogen receptor- α expression in prepubertal heifers: Effects of ovariectomy and growth hormone. *Journal of Dairy Science* 86:2098-2105.
- Capuco, A.V. and Ellis, S. 2004. The mammary gland: developmental changes. *In*: Pond, W. and Bell, A., editors. *Encyclopedia of Animal Science*, New York, NY: Marcel Dekker. p. 603-605.
- Capuco, A.V., and Ellis, S. 2005. Bovine mammary progenitor cells: Current concepts and future directions. *Journal of Mammary Gland Biology and Neoplasia* 10:5-15.

6. Capuco, A.V., Ellis, S., Wood, D.L., Akers, R.M., Garrett, W. 2002. Postnatal mammary ductal growth: three-dimensional imaging of cell proliferation, effects of estrogen treatment and expression of steroid receptors in prepubertal calves. *Tissue and Cell* 34:143-154.

Ellis, S., Capuco, A.V. 2002. Cell proliferation in bovine mammary epithelium: identification of the primary proliferative cell population. *Tissue and Cell* 34:155-163.

Berry, S.D.K., Jobst, P.M., Ellis, S., Howard, R.D., Capuco, A.V., Akers, R.M. 2003. Mammary Epithelial proliferation and estrogen receptor- α expression in prepubertal heifers: Effects of ovariectomy and growth hormone. *Journal of Dairy Science* 86:2098-2105.

Myer, M.J., Capuco, A.V., Ross, D.A., and Van Amburgh, M.E. 2004. Prepubertal Mammary Development in the Bovine: Influence of Nutrition and Age at Puberty. 66th Cornell Nutrition Conference. p 77-95.

Connor, E.E., Wood, D.L., Sonstegard, T.S., Mota, A.F., Bennett, G.L., Williams, J.L., and Capuco, A.V. 2005. Chromosomal mapping and quantitative analysis of estrogen-related receptor alpha-1 (ERR α), estrogen receptors alpha (ER α) and beta (ER β) and progesterone receptor (PR) in the bovine mammary gland. *Journal of Endocrinology* 185:593-603.
7. Connor, E.E., Laiakis, E.C., Fernandes, V.M., Williams, J.L., and Capuco, A.V. 2005. Molecular cloning, expression and radiation hybrid mapping of the bovine deiodinase type II (DIO2) and deiodinase type III (DIO3) genes. *Animal Genetics* 36 (3):240-243.

Capuco, A.V. and Ellis, S. 2004. The mammary gland: developmental changes. *In*: Pond, W. and Bell, A., editors. *Encyclopedia of Animal Science*, New York, NY: Marcel Dekker. p. 603-605.
8. Richards M.P., Poch S.M., McMurtry J.P. 2005. Expression of insulin-like growth factor system genes in liver and brain tissue during embryonic and post-hatch development of the turkey. *Comparative Biochemistry and Physiology Part A Molecular and Integrative Physiology* 141:76-86.

The following gene sequence data was submitted to the NCBI GenBank database and made available to scientists worldwide:

Richards, M.P., Poch, S.M., Clarke, S.M., McMurtry, J.P. 2005. Meleagris gallopavo insulin-like growth factor-II precursor (IGF-II) mRNA, complete cds. GenBank Accession Number AY829236.

Richards, M.P., Poch, S.M., Clarke, S.M., McMurtry, J.P. 2005. Meleagris gallopavo insulin-like growth factor-II precursor (IGF-II) gene, intron 3. GenBank Accession Number AY829237.

Component VII: Pre-Harvest Product Quality

Problem Area VIIa: Interactions of Genetics and Nutrition

1. Lawler, T. L., J. B. Taylor, J. W. Finley, and J. S. Caton. 2004. Effect of supranutritional and organically-bound selenium on performance, carcass characteristics, and selenium distribution in finishing steers. *Journal of Animal Science* 82:1488-1493.

Taylor, J. B. 2005. Time-dependent influence of supranutritional organically bound selenium accumulation in growing wether lambs. *Journal of Animal Science* 83:1186-1193.

Taylor, J. B., J. W. Finley, and J. S. Caton. 2005. Effect of the chemical form of supranutritional selenium on selenium load and selenoprotein activities in virgin, pregnant, and lactating rats. *Journal of Animal Science* 83:422-429.

Problem Area VIIb: Biological Mechanisms Controlling Variation

1. Veiseth, E., Shackelford, S.D., Wheeler, T.L., Koohmaraie, M. 2004. Indicators of tenderization are detectable by 12 h postmortem in ovine longissimus. *Journal of Animal Science* 82:1428-1436.

Veiseth, E., Shackelford, S.D., Wheeler, T.L., Koohmaraie, M. 2004. Factors regulating lamb longissimus tenderness are affected by age at slaughter. *Meat Science* 68:635-640.

2. Kent, M.P., Spencer, M.J., Koohmaraie, M. 2004. Postmortem proteolysis is reduced in transgenic mice overexpressing calpastatin. *Journal of Animal Science* 82:794-801.

Geesink, G.H., Taylor, R.G., Koohmaraie, M. 2005. Calpain 3/p94 is not involved in postmortem proteolysis. *Journal of Animal Science* 83:1646-1652.

3. This work is under review to comply with the terms of the trust agreement.

4. Rhee, M., Wheeler, T.L., Shackelford, S.D., Koohmaraie, M. 2004. Variation in palatability and biochemical traits within and among eleven beef muscles. *Journal of Animal Science* 82:534-550.

Problem Area VIIc: Predicting Quality or Defects

1. Shackelford, S. D., T. L. Wheeler, and M. Koohmaraie. 2003. On-line prediction of yield grade, longissimus muscle area, preliminary yield grade, adjusted preliminary yield grade, and marbling score using the MARC beef carcass image analysis system. *Journal of Animal Science* 81:150-155.

Patent: Haagensen, P., Eger, H, Koohmaraie, M., Shackelford, S. D., Wheeler, T. L. Image analysis systems for grading of meat, predicting quality of meat and/or predicting meat yield of an animal carcass. Patent #6,891,961. May 10, 2005.

Patent: Haagensen, P., Eger, H, Koohmaraie, M., Shackelford, S. D., Wheeler, T. L. Image analysis systems for grading of meat, predicting quality of meat and/or predicting meat yield of an animal carcass. Patent #6,751,364. June 15, 2004.

Obtained USDA-AMS approval for use of the VBG2000 Vision Beef Grading System for prediction of Ribeye Area. December 19, 2003.

Obtained USDA-AMS approval for use of the VBG2000 Vision Beef Grading System for prediction of Official Beef Carcass Yield Grade. August 16, 2005.

2. Wheeler, T. L., D. Vote, J. M. Leheska, S. D. Shackelford, K. E. Belk, D. M. Wulf, B. L. Gwartney, and M. Koohmaraie. 2002. The efficacy of three objective systems for identifying beef cuts that can be guaranteed tender. *Journal of Animal Science* 80:3315-3327.

MARC scientists have worked with the industry and academicians to ensure uniform, accurate application of the MARC slice shear force procedure for measurement of ribeye tenderness. MARC scientists have trained individuals from numerous companies and research institutions to conduct slice shear force. This has allowed several research institutions and private companies to adopt this technology. This technology has allowed a major beef processor and a major retailer to develop and market a billion dollar premium branded beef product line.

3. Wheeler, T. L., D. Vote, J. M. Leheska, S. D. Shackelford, K. E. Belk, D. M. Wulf, B. L. Gwartney, and M. Koohmaraie. 2002. The efficacy of three objective systems for identifying beef cuts that can be guaranteed tender. *Journal of Animal Science* 80:3315-3327.

Shackelford, S.D., Wheeler, T.L., Koohmaraie, M. 2004. Development of optimal protocol for visible and near-infrared reflectance spectroscopic evaluation of meat quality. *Meat Science* 68:371-381.

Shackelford, S.D., Wheeler, T.L., Koohmaraie, M. 2005. On-line classification of US select beef carcasses for longissimus tenderness using visible and near-infrared reflectance spectroscopy. *Meat Science* 69:409-415.

4. Mitchell, A.D., Scholz, A.M., Pursel, V.G. 2003. Prediction of pork carcass composition based on cross-sectional region analysis of dual energy X-ray absorptiometry (DXA) scanning. *Meat Science* 63:265-271.

Mitchell, A.D., Scholz, A.M., Pursel, V.G. 2002. Prediction of the in vivo body composition of pigs based on cross-sectional region analysis of dual energy x-ray absorptiometry (DXA) scans. *Archives of Animal Breeding* 45:5-545.

Mitchell, A.D., Scholz, A.M., Pursel, V.G. 2003. Changes in body composition when young pigs are restricted to near maintenance dietary intake. *International Journal of Body Composition Research* 1:123-128.

Scholz, A.M., Mitchell, A.D., Song, H., Wang, P.C. 2003. ¹³C Nuclear magnetic resonance spectroscopy - A non-invasive in vivo method to measure muscle glycogen metabolism in pigs of different genotypes. *Archives of Animal Breeding* 46:199-211.

Scholz, A.M., Mitchell, A.D. 2003. Protein, fat, and bone mineral deposition rates in growing pigs (birth to 90 kg) of different RyR1 genotypes studied by dual energy x-ray absorptiometry. In: *Progress in Research on Energy and Protein Metabolism*: (W.B. Souffrant and C.C. Metges, Eds.). EAAP Scientific Series v.109:543-548.

Mitchell, A.D., Scholz, A.M. 2004. Body Composition: Indirect Measurements. In: Pond, W., Bell, A., editors. *Encyclopedia of Animal Science*, New York, NY: Marcel Dekker, Inc. p. 166-169.

Component VIII: Integrated Systems

Problem Area VIIIa: User Information Packages

1. Williams, C. B., and T. G. Jenkins. 2003. A dynamic model of metabolizable energy utilization in growing and mature cattle. I. Metabolizable energy utilization for maintenance and support metabolism. *Journal of Animal Science* 81:1371-1381.

Williams, C. B., and T. G. Jenkins. 2003. A dynamic model of metabolizable energy utilization in growing and mature cattle. II. Metabolizable energy utilization for gain. *Journal of Animal Science* 81:1382-1389.

Williams, C. B., and T. G. Jenkins. 2003. A dynamic model of metabolizable energy utilization in growing and mature cattle. III. Model evaluation. *Journal of Animal Science* 81:1390-1398.

Williams, C. B. 2005. Technical Note: A dynamic model to predict the composition of fat-free matter gains in cattle. *Journal of Animal Science* 83:1262-1266.

2. Mertens, D.R., Getz, J.E. 2004. Developing consistent relationships among fiber fractions for uniform alfalfa hay quality guidelines. *Journal of Dairy Science*. 87(Suppl.1):292.

The following websites document the use of the results of this research:

http://www.ams.usda.gov/mnreports/GX_GR310.txt

http://www.ams.usda.gov/mnreports/OX_GR310.txt

3. Mertens, D.R. 2005. Particle size, fragmentation index, and effective fiber: Tools for evaluating the physical attributes of corn silages. In Proceedings of the 4-State Dairy Nutrition and Management Conference. Midwest Plan Service, Iowa State Univ., Ames. p. 211-220.
4. Mertens, D.R., K. Bolton, and M. Jorgensen. 2005. Checking dry matters made easy. Hoard's Dairyman 150(11):444-445.

NATIONAL PROGRAM 105 – ANIMAL WELL-BEING AND STRESS CONTROL SYSTEMS

Problem Area Ia: Scientific Measures of Well-Being

1. "Animal feed composition and methods using the same". Patent granted. U.S. Patent Application 10/192,762. Purdue Ref. NO.: P-00106.00.US. Licensing agreement between Purdue Research Foundation and Natural Chem. Group, LLC.
2. Not all aspects of this accomplishment have advanced to the point for an over-arching peer-reviewed publication.
3. Eicher, S.D., K.A. McMunn, H.M. Hammon, and S.S. Donkin. 2004. Toll-like receptors 2 and 4, and acute phase cytokine gene expression in dexamethasone and growth hormone treated dairy calves. Vet. Immunol. Immunopathology 98:115-125.
4. Strauch, T.A., J.A. Carroll, T. J. Fangman, C.E. Wiedmeyer, and A. K. Hamback. 2004. The acute phase response of *Escherichia coli* challenged pigs exhibiting a febrile response in the absence of elevated TNF- α . Journal of Animal and Veterinary Advances 3(4):227-238.
5. This research has not progressed to the point of a peer-reviewed article.

Problem Area Ib: Adaptation and Adaptedness

1. Cheng, H.W., S.D. Eicher, Y. Chen, P. Singleton, and W.M. Muir. 2001. Effect of genetic selection for group productivity and longevity on immunological and hematological parameters of chickens. Poultry Science 80:1079-1086.

Cheng, H.W., G. Dillworth, P. Singleton, Y. Chen, and W.M. Muir. 2001. Effects of group selection for productivity and longevity on blood concentrations of serotonin, catecholamine and corticosterone of laying hens. Poultry Science 80:1278-1285.

Cheng, H.W., P. Singleton, and W.M. Muir. 2002. Social stress in laying hens: Differential dopamine and corticosterone responses following intermingling of different genetic strain chickens. Poultry Science 81:1265-1273.

Cheng, H.W., P. Singleton, and W.M. Muir. 2003. Social stress differentially regulates neuroendocrine responses in laying hens: I. Genetic basis of dopamine responses under three different social conditions. Psychoneuroendocrinology 28:597-611.

Cheng, H.W., P. Singleton, and W.M. Muir. 2003. Social stress in laying hens: Differential effect of genetic-environmental interactions on plasma dopamine concentrations and adrenal function in genetically selected chickens. Poultry Science 82:192-198.

Cheng, H.W. and W.M. Muir. 2004. Chronic social stress differentially regulates neuroendocrine responses in laying hens: II. Genetic basis of adrenal responses under three different social conditions. *Psychoneuroendocrinology* 97:961-971.

Cheng, H.W. and W.M. Muir. 2005. Genetic selection in poultry: Physiological factors associated with production and survivability. *World's Poultry Science Journal* 61:383-398.

Problem Area Ic: Social Behavior and Spacing

1. Freire, R. and H.W. Cheng. 2004 Experience-dependent changes in the hippocampus of domestic chicks: a model for spatial memory. *European Journal of Neuroscience* 20:1065-1068.

Freire R., H.W. Cheng, and C. Nicol. 2004. Development of spatial memory in occlusion-experienced domestic chicks. *Animal Behavior* 67:141-150.

2. A new area of work not advanced far enough for a peer-reviewed publication.
3. This project has been completed and a manuscript has been submitted to the *Journal of Animal Science*.

Problem Area Ie: Evaluate Practices and Systems to Improve Well-Being

1. Koch et al., 2005. Melengestrol acetate in experimental diets as an effective alternative to induce a decline in egg production and reversible regression of the reproductive tract in laying hens I. Determining an effective concentration of MGA. *Poultry Science* (In Press).

Koch et al., 2005. Melengestrol acetate as an effective alternative to induce a decline in egg production and reversible regression of the reproductive tract in laying hens II. Effects on post-molt egg quality. *Poultry Science* (In Press).

Patent Application. Ovarian Regression and Recrudescence in the Hen Using Melengestrol Acetate, 0174.03. D.C. Lay Jr. and M. E. Wilson.

2. Sorrells, A.D., S. D. Eicher, K. A. Scott, M. J. Harriss, E. A. Pajor, D. C. Lay, Jr., and B. T. Richert. 2005. Post-natal behavioral and physiological responses of piglets from sows housed individually or in groups during gestation. *Journal of Animal Science* (Accepted 10-21-05).
3. Grellner, G.F., T.J. Fangman, J.A. Carroll and C.E. Wiedmeyer. 2002. Using serology in combination with acute phase proteins and cortisol to determine stress and immune function of early-weaned pigs. *Journal of Swine Health and Production* 10(5):199-204.

Gaines, A.M., J.A. Carroll, G.L. Allee, and G.F. Yi. 2002. Pre- and postweaning performance of pigs injected with dexamethasone at birth. *Journal of Animal Science* 80:2255-2262.

Gaines, A.M., J.A. Carroll, and G.L. Allee. 2003. Nursery performance of pigs injected with exogenous glucocorticoids at weaning. *Journal of Animal and Veterinary Advances* 2(1):22-26.

Seaman-Bridges, J.S., J.A. Carroll, T.J. Safranski and E.P. Berg. 2003. Short- and long-term influence of perinatal dexamethasone treatment on swine growth. *Domestic Animal Endocrinology* 24(3):193-208.

4. Branton, S.C., Simmons, J.D., Lott, B.D., Miles, D.M. 2001. Chick mortality associated with elevated water lines and consumption of wet litter. *Journal of Applied Poultry Research* 10: 427-430.

Lott, B.D., May, J.D., Simmons, J.D., Branton, S.L. 2001. The effect of nipple height on broiler performance. *Poultry Science* 80: 408-410.

Miles, D.M., Lott, B.D., Branton, S.L., Simmons, J.D. 2004. Development of a "water stick" to measure nipple waterer flow rates. *Journal of Applied Poultry Research* 13:258-262.

5. Research is in progress.

6. Touchette, K.J., J. A. Carroll, G. L. Allee, R. L. Matteri, C. J. Dyer, L. A. Beausang, and M. E. Zannelli. 2002. Effect of spray-dried plasma and lipopolysaccharide exposure on weaned pigs: I. Effects on the immune axis of weaned pigs. *Journal of Animal Science* 80:494-501.

Carroll, J.A., K. J. Touchette, R. L. Matteri, C. J. Dyer, and G. L. Allee. 2002. Effect of spray-dried plasma and lipopolysaccharide exposure on weaned pigs: II. Effects on the hypothalamic-pituitary-adrenal axis of weaned pigs. *Journal of Animal Science* 80:502-509.

Carroll, J.A., A.M. Gaines, J.D. Spencer, G.L. Allee, H.G. Kattesh, and M.E. Zannelli. 2003. Effect of menhaden fish oil supplementation and lipopolysaccharide exposure on nursery pigs: I. Effects on the immune axis when fed diets containing spray-dried plasma. *Domestic Animal Endocrinology* 24:341-351.

Gaines, A.M., J.A. Carroll, G.F. Yi, G.L. Allee, and M.E. Zannelli. 2003. Effect of menhaden fish oil supplementation and lipopolysaccharide exposure on nursery pigs: II. Effects on the immune axis when fed simple or complex diets containing no spray-dried plasma. *Domestic Animal Endocrinology* 24:353-365.

Frank, J.W., J.A. Carroll, G.L. Allee, and M.E. Zannelli. 2003. The effect of thermal environment and spray-dried plasma on the acute-phase response of pigs challenged with lipopolysaccharide. *Journal of Animal Science* 81:1166-1176.

7. Salfen, B.E., J.A. Carroll, D.H. Keisler, and T.A. Strauch. 2004. Effects of exogenous ghrelin on feed intake, weight gain, behavior, and endocrine parameters in weanling pigs. *Journal of Animal Science* 82:1957-1966.
8. Simmons, J.D., Miles, D.M., Lott, B.D. 2003. The effects of high air velocity on broiler performance. *Poultry Science*. 82:232-234.

Dozier III, W.A., Lott, B.D., Branton, S.L. 2005. Growth responses of male broilers subjected to increasing air velocities at high ambient temperatures and a high dewpoint. *Poultry Science* 84:962-966.

Dozier III W.A., Lott, B.D., and Branton S.L. 2005. Live performance of male broilers subjected to constant or increasing air velocities at moderate temperatures with a high dewpoint. *Poultry Science* 84:1328-1331.

9. The results have been presented at various scientific meetings and two publications are being prepared.
10. Carroll, J.A., T.J. Fangman, A.K. Hambach and C.E. Wiedmeyer. 2004. The acute phase response in pigs experimentally infected with *Escherichia coli* (*E. coli*) and treated with systemic bactericidal antibiotics. *Livestock Production Science* 85:35-44.
11. Roush, W. B., Boykin, D. L., Branton, S. L. 2004. Optimization of phase feeding of starter, grower and finisher diets for male broilers by mixture experimental design: 48-day production period. *Poultry Science*. 83:1264-1275.
- Roush, W. B. and Branton, S. L. 2005. A comparison of fitting models with a genetic algorithm and nonlinear regression. *Poultry Science* 84:294-502.

Problem Area If: Bioenergetic Criteria for Environmental Management

1. Eigenberg, R.A., Brown-Brandl, T.M., Nienaber, J.A. 2002. Development of a respiration rate monitor for swine. *Trans. ASAE* 45(5):599-1603.

- Freetly, H.C., Nienaber, J.A., Brown-Brandl, T.M. 2002. Relationship between aging and nutritional controlled growth rate on heat production of ewe lambs. *Journal of Animal Science* 80:2759-2763.
- Brown-Brandl, T.M., Nienaber, J.A., Eigenberg, R.A., Freetly, H.C., Hahn, G.L. 2003. Thermoregulatory responses of feeder cattle. *J. Therm. Biol.* 28:149-157.
- Freetly, H.C., Nienaber, J.A., Brown-Brandl, T.M. 2003. Relationships between aging and nutritionally controlled growth rate on heat production of heifers. *Journal of Animal Science* 81:1847-1852.
- Kerr, B.J., Yen, J.T., Nienaber, J.A., Easter, R.A. 2003. Influences of dietary protein level, amino acid supplementation and environmental temperature on performance, body composition, organ weights and total heat production of growing pigs. *Journal of Animal Science* 81:1998-2007.
- Brown-Brandl, T.M., Yanagi, T., Xin, H., Gates, R.S., Bucklin, R., Ross, G.S. 2003. A new telemetry system for measuring core body temperature in livestock and poultry. *Applied Engineering in Agriculture* 19(5):583-589.
- Yen, J.T., Varel, V.H., Nienaber, J.A. 2004. Metabolic and microbial responses in western crossbred and Meishan growing pigs fed a high-fiber diet. *Journal of Animal Science* 82:1740-1755.
- Brown-Brandl, T.M., Nienaber, J.A., Xi, H., Gates, R.S. 2004. A literature review of swine heat production. *Trans. ASAE* 47(1):259-270.
- Brown-Brandl, T.M., Eigenberg, R.A., Hahn, G.L., Nienaber, J.A., Mader, T.L., Spiers, D.E., Parkhurst, A.M. 2005. Analyses of thermoregulatory responses of feeder cattle exposed to simulated heat waves. *International Journal of Biometry* 49:285-296.
- Eigenberg, R.A., Brown-Brandl, T.M., Nienaber, J.A., Hahn, G.L. 2005. Dynamic response indicators of heat stress in shaded and non-shaded feedlot cattle: part 2 predictive relationships. *Biosystems Engineering* 91(1):111-118.
- Brown-Brandl, T.M., Eigenberg, R.A., Nienaber, J.A., Hahn, G.L. 2005. Dynamic response indicators of heat stress in shaded and non-shaded feedlot cattle: part.1. Analysis of indicators. *Biosystems Engineering* 90(4):451-462.
- Brown-Brandl, T.M., Jones, D.D., Woldt, W.E. 2005. Evaluating modeling techniques for cattle heat stress prediction. *Biosystems Engineering* 91(4):513-524.

APPENDIX 2 – Listing of Individual Appropriated CRIS Projects by Geographic Location

NATIONAL PROGRAM 101 – FOOD ANIMAL PRODUCTION

Fort Collins, Colorado -- National Center for Genetic Resources Preservation

- [National Animal Germplasm Program \(NAGP\)](#)

CRIS Project Title: *National Animal Germplasm Program* (NP 101- Component II)

SY (2.0): H. Blackburn (Lead), P. Purdy

Appropriated Annual Funding: \$679,121

Related Sibling CRIS Projects:

- Support of At Risk Avian Genetic Stocks and National Assessment of Avian Genetic Resources – Univ. California, Davis. 5402-31000-002-01S
- Preserve Warhill Ram Germplasm - Colorado State University. 5402-31000-002-05S
- Preservation of Goat Germplasm – Prairie View A&M. 5402-31000-001-07S
- Determine the Genetic Similarity Between Two Separate Lines of Hereford Cattle – Colorado State University. 5402-31000-002-03S
- Preservation of Goat Germplasm – Sul Ross University. 5402-31000-002-06S
- Collection, Cryopreservation and Evaluation of Different Poultry Lines – Colorado State University. 5402-31000-002-07S
- Population Status of Livestock Genetic Resources in the United States – American Livestock Breeds Conservancy. 5402-31000-002-02S
- Development of a National Collection of Swine – Purdue University. 5402-31000-002-04S
- Economic Evaluation of the Exchange of Animal Genetic Resources – Williams College. 5402-31000-002-10G
- Preservation of Swine Germplasm – Purdue University. 5402-31000-002-11S
- Sheep and Goat Embryo Cryopreservation for Conservation of Genetic Resources – Louisiana State University. 5402-31000-002-09S
- The Effect of Holding Time on Ram Semen Fertility – University of Wyoming. 5402-31000-002-08S

Species Impacted: Beef Cattle, Dairy Cattle, Swine, Poultry, Sheep, Goats, and Aquaculture (catfish, trout, salmon, oysters, striped bass, etc)

Listing of Project Objectives:

- 1) Understanding population structures and demographic changes in population size, location and number of producers raising specific breeds.
- 2) Acquiring germplasm (semen, embryos, ova and DNA) for storage at the National Center for Genetic Resources Preservation.
- 3) Evaluation, development and implementation of cryopreservation protocols for germplasm preservation in liquid nitrogen.
- 4) Development of an information system that links genetic preservation (ex-situ and in-situ) management activities of animal populations.
- 5) Develop, coordinate and assist species committees in setting priorities and procedures for ex-situ and in-situ management of genetic resources.

- Brooksville, Florida – Subtropical Ag. Research Station**
- [Evaluation of Beef Cattle Germplasm for the Subtropics of the United States](#)

CRIS Project Title: *Evaluation of Beef Cattle Germplasm for the Subtropics of the U.S.*
(NP 101- Components I, II, III, V)

SY (2.60): C. Chase (Lead), D. Riley, S. Coleman

Appropriated Annual Funding: \$731,430

Related Sibling CRIS Projects:

6619-31630-002-00D Improving Fertility of Heat-Stressed Dairy Cattle

Species Impacted: Beef Cattle

Listing of Project Objectives:

- 1) Complete a progeny evaluation of Brahman sires to determine the degree of additive genetic variance for carcass yield, quality, and palatability characteristics, and determine the association of these phenotypes with quantitative trait loci.
- 2) Evaluate the Romosinuano (tropical *Bos taurus*) with Angus (temperate *Bos taurus*) and Brahman (tropical *Bos indicus*) breeds in a diallel mating scheme to:
 - a) determine among breeds of cows used in the diallel the effect of two nutritional systems on cow productivity, including reproductive efficiency and calf performance,
 - b) determine heterosis and direct and maternal breed effects on preweaning and weaning traits of calves produced in the diallel,
 - c) determine in heifers produced by diallel matings the effect of breed type on postweaning growth, circulating concentrations of metabolites and hormones, puberty, and pregnancy,
 - d) determine in steers produced by diallel matings the effect of breed type on stress, response to shipment, postweaning growth, feedlot performance, and carcass yield, quality, and palatability characteristics.

Athens, Georgia -- Richard B. Russell Research Center – Animal Physiology Unit

- [Role of the Adipose Tissue-Brain-Pituitary Axis in Growth and Reproduction](#)

CRIS Project Title: *Role of the Adipose Tissue-Brain-Pituitary Axis in Growth and Reproduction*
(NP 101-Components I, VI, VII)

SY (2.0): C. Barb (Lead), G. Hausman

Appropriated Annual Funding: \$615,290

Related Sibling CRIS Projects: None

Species Impacted: Swine

Listing of Project Objectives:

- 1) Identify gene expression and protein products associated with development and function of the hypothalamus, pituitary, follicle and corpus luteum during pubertal development, the estrous cycle and subsequent changes in adiposity in swine.
- 2) Determine the effects of nutrition and fat cell secretory products on the expression of hypothalamic genes and proteins that regulate luteinizing hormone and growth hormone secretory pathways in swine.
- 3) Determine mechanisms that regulate gene and protein expression during preadipocyte proliferation and differentiation during growth and development in swine.

Dubois, Idaho – U.S. Sheep Experiment Station

- [Integrated Systems for Increasing Production Efficiency of Sheep](#)

CRIS Project Title: *Integrated Systems for Increasing Production Efficiency of Sheep*
(NP 101-Components I, II, III, V, VII)

SY (3.70): G. Lewis (Lead), J. Stellflug, J. Taylor, Vacant, Vacant

Appropriated Annual Funding: \$1,430,476

Related Sibling CRIS Projects:

5364-31000-007-01R A Ram Model of Neuroendocrine Function Objectives

5364-31000-007-03N Splanchnic and Hind Limb Metabolism of Selenium and Methionine in Infection Stressed Ewes

5364-31000-007-04S Characterization of Sheep Breeds and Development of Composite Lines Suitable for Range Environments

5364-31000-007-05R The Influence of Maternal Environment on Fetal Development, and Progeny Weight, Growth and Health

5364-31000-007-08N Improving Reproductive Performance of Sheep

Species Impacted: Sheep

Listing of Project Objectives:

- 1) Develop and use advanced reproductive technologies for efficient production of genetically superior sheep.
- 2) Further develop and validate nonsurgical, transcervical artificial insemination procedures for sheep.
- 3) Determine whether manipulating uterine immune functions at strategic points in the reproductive cycle will reduce the incidence of uterine bacterial contamination and prevent subsequent reductions in pregnancy and lambing rates.
- 4) Determine whether strategic nutrient intervention will mitigate the negative effects of stressors, which are associated with producing sheep for range environments, on growth, health, and reproductive processes and on skeletal muscle nutrient composition.
- 5) Develop and validate methods for characterizing sexual performance of rams, which are suitable for range environments, and reproductive efficiency of their daughters.
- 6) Characterize sheep breeds and develop composite sire lines that are suitable for terminal crosses in range environments.
- 7) Evaluate different biological types of sheep for reproductive efficiency, rate and efficiency of growth, carcass composition, meat quality, and mature size.
- 8) Use quantitative genetic methods to identify physical and physiological criteria for enhancing reproductive efficiency and other economically important traits, including meat quality, quantity, and flavor.

Lexington, Kentucky -- Forage-Animal Production Research Unit

- [Improved Forage Livestock Production](#)

CRIS Project Title: *Enhance Forage-Based Livestock Production Systems*
(NP 101- Components I, V)

SY (6.0): J. Strickland (Lead), G. Aiken, J. Klotz, I. Kagan, R. Dinkins

Appropriated Annual Funding: \$2,511,393

Related Sibling CRIS Projects: None

Species Impacted: Beef Cattle, Sheep, Goats, Horses

Listing of Project Objectives:

- 1) Improve the persistence, productivity, and quality of tall fescue and clover forages.
- 2) Define the toxicodynamics and toxicokinetics of the alkaloids found in endophyte-infected tall fescue.
- 3) Identify plant chemical and/or physical factors that affect forage selection, intake, and utilization in animals grazing in mixed forage environments.
- 4) Develop sustainable grazing systems for optimal forage-animal production that capitalize on mixed forage systems and strategic nutrient supplementation.
- 5) Determine the etiology of Mare Reproductive Loss Syndrome (MRLS).

Note: This is a new research unit that has not yet been through the ARS OSQR process and will be split between NPs 101 and 205 in the upcoming cycle. All funding and SY for the unit are shown.

Beltsville, Maryland – Beltsville Agricultural Research Center
Animal Improvement Programs Laboratory

- [Improving Genetic Prediction of Economic Merit of Dairy Animals](#)

CRIS Project Title: *Improving Prediction of Economic Merit of Dairy Animals*
(NP 101- Components I, II, III, IV)

SY (5.1): D. Norman (Lead), J. Cole, M. Kuhn, G. Wiggans, C. Van Tassell, P. VanRaden

Appropriated Annual Funding: \$2,500,794

Related Sibling CRIS Projects:

[Improving Identification, Reproduction, and Health in Dairy Cattle](#)
[Improving Identification, Reproduction, and Health in Dairy Cattle](#)
[Improving Identification, Reproduction, and Health in Dairy Cattle](#)
[Improving Identification, Reproduction, and Health in Dairy Cattle](#)
[Improving Reproduction and Health in Dairy Cattle Through Use of on-Farm Databases](#)
[Improving Genetic Prediction of Economic Merit of Dairy Animals](#)
[Estimating Economic Contribution of Daughter Pregnancy Rate to Lifetime Net Merit](#)
[Effects of Heat Stress on Reproduction, Production, and Survival of Holsteins and Jerseys Or Holsteins and Brown Swiss in the Same Herd](#)
[Improving Fitness in Dairy Cattle by Facilitating the Initiation of a National Research Database for Health Traits](#)
[Estimating Daily Milk, Component Yield, and SCS from Individual Milking Data](#)
[Improving Prediction of Dairy Bull Genetic Merit Considering Heat Stress by Geographical Region](#)
[Genetic and Environmental Parameters for Yield Traits Across Varying Herd Sizes, Production Levels and Lactation Number](#)
[Effects of Heat Stress on Parameters of Lactation Curves for Cows of Different Breeds](#)
[Enhanced Productive Life in Merit Indexes](#)
[The National Dairy Genetic Evaluation Program](#)
[Development and Enhancement of Genetic Evaluations for Calving Traits](#)
[Improvements in Genetic Evaluation Software for Calving Ease](#)
[Use of Single Nucleotide Polymorphisms to Verify Parentage](#)

Species Impacted: Dairy Cattle

Listing of Project Objectives:

- 1) Maintain, enhance, and expand development of a national dairy database of identification (domestic and foreign animals), production (yield, milk composition), fitness (conformation, longevity), reproduction (dystocia, fertility), and health (mastitis, disease resistance) traits to support research on dairy animal genetics and management; provide data to stakeholders and other researchers submitting proposals compatible with industry guidelines.
- 2) Improve the accuracy of genetic evaluations for yield traits through inclusion of additional data, appropriate weighting of deviant data, and improved methodology.
- 3) Identify and adapt appropriate statistical tools for analysis of complex dairy pedigrees and develop bioinformatic tools to automate data processing in support of quantitative trait locus detection, marker testing, and mapping methods.
- 4) Improve genetic rankings for overall economic merit by development of evaluations for additional traits (calving ease with maternal effects included, fertility, and other health and management variables) and by determining more precisely the economic values of traits in the index through improved profit functions from updated incomes and expenses associated with each trait available for selection.
- 5) Characterize dairy industry programs and practices (milk recordkeeping associations, breed registry societies, artificial-insemination organizations) to document status and changes in data collection and use and in phenotypic and genetic trends (evaluation summaries and stability).

Beltsville, Maryland – Beltsville Agricultural Research Center

Biotechnology and Germplasm Laboratory

- [Identification and Manipulation of Genetic Factors to Enhance Disease Resistance in Dairy Cattle](#)
 - [Swine Germplasm Preservation, Propagation, and Embryo Developmental Competence](#)
 - [Analysis of Sperm Storage Mechanisms in Poultry](#)
 - [Proteomic Analysis of Factors Regulation Egg Production in the Hen](#)

**CRIS Project Title: *Identification and Manipulation of Genetic Factors to Enhance Disease Resistance in Dairy Cattle*
(NP 101- Components I, III, IV)**

SY (3.85): R. Wall (Lead), L. Blomberg, D. Donovan, N. Talbot, K. Zuelke

Appropriated Annual Funding: \$2,515,400

Related Sibling CRIS Projects:

1265-31000-080-01S, 1265-31000-080-08S (University of Illinois and University of Connecticut SCAs)

Species Impacted: Dairy Cattle

Listing of Project Objectives:

- 1) Produce and characterize mouse models designed to demonstrate the efficacy of using genetic engineering as a tool to increase resistance to mastitis. Mammary gland function and physical properties of milk from lysostaphin transgenic mice will be characterized. Transgenic mice containing newly cloned rainbow trout lysozyme gene will be produced and characterized as will double lysostaphin / lysozyme mice transgenic. Use of lactoferrin regulatory element will be evaluated as a switch to turn on antimicrobial peptides as needed.
- 2) Produce and characterize transgenic cows carrying mammary gland targeted lysostaphin. When warranted, based on mouse model studies, transgenic cows carrying other antimicrobial proteins will be produced and characterized.
- 3) Improve the efficiency of producing transgenic nuclear transfer progeny. Protein expression of nuclear transfer-, in vitro-, and parthenogenic embryos will be compared to identify conditions in somatic cell nuclear transfer that can be altered to better support development to term.

**CRIS Project Title: *Swine Germplasm Preservation, Propagation, and Embryo Developmental Competence*
(NP 101- Components I, II, IV)**

SY (2.0): K. Zuelke (Lead), L. Blomberg, D. Guthrie

Appropriated Annual Funding: \$885,206

Related Sibling CRIS Projects: None

Species Impacted: Swine

Listing of Project Objectives:

- 1) To analyze the transcriptome of in vivo produced pre-implantation pig embryos using Serial Analysis of Gene Expression (SAGE) and other complementary genomics tools to identify and quantitate the relative abundance of differentially expressed genes within and between critical developmental stages.
- 2) To increase developmental competence of in vitro produced (IVP) porcine embryos by integrating results of functional genomic analyses of early embryonic development into embryo production systems.
- 3) To develop and improve methods for long-term preservation, hypothermic storage and transfer of swine germplasm and embryos.

CRIS Project Title: *Analysis of Sperm Storage Mechanisms in Poultry*
(NP 101- Components I, II, IV)

SY (2.1): M. Bakst (Lead), J. Long, K. Zuelke

Appropriated Annual Funding: \$990,432

Related Sibling CRIS Projects: None

Species Impacted: Poultry

Listing of Project Objectives:

- 1) Determine how and when poultry sperm lose functional competence during liquid and cryogenic storage and use as a basis for developing successful sperm storage methods in vitro.
- 2) Determine the influence of sperm phenotype (mobility, fertilizing capability) on liquid and cryogenic storage of turkey sperm, as well as phenotypic traits of economic importance in breeder males (feed conversion, body conformation, liveability).
- 3) Increase the efficacy of oviductal sperm transport to the sperm storage tubule (SST) and the site of fertilization by elucidating the cellular and molecular mechanisms regulating sperm selection and transport in the oviduct.
- 4) Determine the molecular basis of sperm subsistence in the sperm storage tubules using serial analysis of gene expression (SAGE) and developing an in vitro model (tissue explants) to elucidate molecular and cellular events regulating prolonged oviductal sperm storage and sustained fertility in poultry.
- 5) Develop capability to produce transgenic poultry isolated testicular germ cells, transfected and transferred to recipient sterile male. This work has been approved by the NPL as an addendum and is part of an anticipated multinational cooperative agreement.

CRIS Project Title: *Proteomic Analysis of Factors Affecting Regulation of Egg Production in the Hen*
(NP 101- Components I, IV)

SY (1.2): J. Proudman (Lead), K. Zuelke

Appropriated Annual Funding: \$805,402

Related Sibling CRIS Projects: None

Species Impacted: Poultry

Listing of Project Objectives:

- 1) Implement state-of-the-art techniques for proteomic analysis at the cellular level, including sample preparation, labeling, instrumentation and computer analysis.
- 2) Identify cellular factors in the brain and pituitary that enhance or terminate egg production. Prior research has identified specific brain nuclei and pituitary cells that likely regulate incubation behavior, a major cause of poor reproduction in the turkey hen.
- 3) Develop genomic tools to identify breeders with superior reproductive potential and/or therapeutic intervention to modify reproductive efficiency.
- 4) Develop management strategies for prolonging the reproductive season of turkey breeder hens.
- 5) Provide hormones, antibodies and techniques to scientists and industry for the improvement of poultry production efficiency.

Beltsville, Maryland – Beltsville Agricultural Research Center

Bovine Functional Genomics Laboratory

- [Identification, Validation and Fine-Mapping of Quantitative Trait Loci in Dairy Cattle](#)
 - [Functional Genomics of Dairy Production](#)
- [Physiological Regulation and Functional Genomics of Ruminant Nutrient Metabolism](#)
 - [Development of Bioinformatics Tools for Livestock](#)

CRIS Project Title: *Identification, Validation, and Fine-Mapping of Quantitative Trait Loci in Dairy Cattle*
(NP 101- Components II, III, IV)

SY (1.7): T. Sonstegard (Lead), R. Li, C. Van Tassell

Appropriated Annual Funding: \$764,132

Related Sibling CRIS Projects:

1265-3100-081-04R: A functional genomics approach to define relationships between stress & immunity in cattle.

1265-3100-081-05N: Validation of parasite indicator QTL for cattle in a sheep resource population.

Species Impacted: Dairy Cattle

Listing of Project Objectives:

- 1) Fine-map and characterize previously identified QTL, such as QTL affecting protein percentage on BTA6 and other QTL affecting dairy form on BTA27.
- 2) Develop high-resolution comparative and BAC contig maps of regions containing QTLs, such as those on BTA6 and BTA27, and map bovine ESTs.
- 3) Identify new QTL affecting reproduction, milk production, conformation and health traits from novel resource populations, such as new families with extreme phenotypes and more contemporary generations of previously studied Holstein families.
- 4) Identify and adapt appropriate statistical tools for analysis of complex dairy pedigrees and develop bioinformatic tools to automate data processing in support of QTL detection and mapping methods.

CRIS Project Title: *Functional Genomics of Dairy Production*
(NP 101- Components I, III, IV)

SY (1.2): A. Capuco (Lead), E. Connor, R. Li, T. Sonstegard

Appropriated Annual Funding: \$769,585

Related Sibling CRIS Projects:

1265-31000-086-01S; Photoperiod effects on body and mammary gland growth of dairy heifers

Species Impacted: Dairy Cattle

Listing of Project Objectives:

- 1) Characterize bovine expression libraries from tissues of mammary gland and gastrointestinal tract for purpose of gene sequence discovery. Bovine expressed sequence tags (EST) provide a basis for comparative functional genomics and for the design and application of microarrays to interrogate the transcriptome.
- 2) Develop a high-density bovine cDNA microarray for use in studies of mammary gland gene expression.
- 3) Survey the mammary gland transcriptome and ascribe function to genes that are significantly modulated during changes in animal physiology related to mammary growth, secretory activity and mastitis.

CRIS Project Title: *Physiological Regulation and Functional Genomics of Ruminant Nutrient Metabolism*
(NP 101- Components IV, V, VI)

SY (1.5): R. Baldwin (Lead), E. Connor

Appropriated Annual Funding: \$1,205,704

Related Sibling CRIS Projects: None

Species Impacted: Beef Cattle, Dairy Cattle

Listing of Project Objectives:

- 1) Identify metabolic (enzyme pathways, nutrient transport) and physiological functions (growth factor expression and receptors) that are regulators of nutrient economy within the animal.
- 2) Develop and use animal models (natural species differences, genetic variation, perturbation with exogenous compounds, and/or level of production) to assess the extent to which differential expression of critical genes can result in altered nutrient economy.
- 3) Develop and characterize cDNA libraries from ruminant intestine and stomach complex to facilitate gene discovery and delineate critical pathways relating to nutrient use and visceral tissue growth.

CRIS Project Title: *Development of Bioinformatic Tools for Livestock*
(NP 101- Components III, IV)

SY (1.45): C. Van Tassell (Lead), G. Liu

Appropriated Annual Funding: \$481,207

Related Sibling CRIS Projects:

1265-31000-090-01S - Application of Bioinformatics to Livestock Genomes

Species Impacted: Dairy Cattle, Beef Cattle, Poultry

Listing of Project Objectives:

- 1) Development of an integrated database and data processing platform for genetic marker data.
- 2) Development of a sequence processing and analysis pipeline and associated tools.
- 3) Development of tools to facilitate functional genomics and proteomics research.

Beltsville, Maryland – Beltsville Agricultural Research Center

Growth Biology Laboratory

- [Endocrine and Immune Mechanisms Affecting Growth in Young Cattle](#)
- [Genetic Regulation of Feed Intake and Energy Balance in Poultry and Swine](#)
- [Functional Genomics of Growth and Development of Swine and Poultry](#)

**CRIS Project Title: *Genetic Regulation of Feed Intake and Energy Balance in Poultry and Swine*
(NP 101- Components I, IV, V, VI)**

SY (3.0): T. Ramsay (Lead), T. Caperna, J. McMurtry, M. Richards

Appropriated Annual Funding: \$925,073

Related Sibling CRIS Projects: None

Species Impacted: Poultry, Swine

Listing of Project Objectives:

- 1) Determine the effects of varying levels of feed intake and/or diet composition on the expression of key regulatory genes and gene products (glucagon-like peptide 1 [GLP-1], leptin, leptin receptor, and uncoupling proteins) that influence appetite and energy balance.
- 2) Investigate the interaction of leptin and GLP-1 with other key metabolic hormones (insulin, glucagon, thyroid hormones) on these key regulatory genes for energy metabolism.
- 3) Determine the influence of coccidiosis and environmental temperature on the expression of these key regulatory genes for appetite and energy balance regulation.
- 4) Identify the mechanisms of action for leptin on biochemical pathways of energy metabolism in hepatocytes and adipocytes; e.g., sodium- potassium ATPases, synthesis and secretion of acute phase proteins, lipid and glucose metabolism.

**CRIS Project Title: *Functional Genomics of Growth and Development of Swine and Poultry*
(NP 101- Components IV, V, VI)**

SY (3.2): M. Richards (Lead), J. McMurtry, A. Mitchell, R. Rosebrough, R. Wall

Appropriated Annual Funding: \$1,718,454

Related Sibling CRIS Projects: None

Species Impacted: Poultry, Swine

Listing of Project Objectives:

- 1) Characterize the regulation of lean/fat tissue accretion and musculoskeletal development in poultry by determining the roles of both novel genes identified by: (a) EST sequencing, cDNA microarrays, differential display, and (b) QTL searches in reference populations, as well as (c) known genes including uncoupling proteins, insulin-like growth factors, myostatin, lipogenic/lipolytic enzymes (acetyl-CoA carboxylase, fatty acid synthase, lipoprotein lipase, and malic enzyme).
- 2) Manipulate the expression of genes in swine including: (a) myostatin, (b) essential amino acid biosynthetic enzymes, and their regulatory partners for increased feed efficiency, increased muscle mass, reduced carcass fat and nitrogen waste.
- 3) Utilize methods of indirect measurements, with a primary emphasis on the use of dual-energy X-ray absorptiometry (DEXA), to determine the effects of gene manipulation on body composition, specifically the fat/lean content and bone mineralization over a wide range of body sizes.

CRIS Project Title: *Endocrine and Immune Mechanisms Affecting Growth in Young Cattle*
NP 101-Components V, VI)

SY (2.0): T. Elsasser (Lead), C. Li

Appropriated Annual Funding: \$1,221,857

Related Sibling CRIS Projects: None

Species Impacted: Beef cattle

Listing of Project Objectives:

- 1) Identify, characterize, and assess the utility of tissue specific enzymes, genes, and ubiquitinated and nitrated-proteins (collectively referred to here as endogenous “chemically modified proteins”) as (a) preemptive biomarkers and indicators of host reaction to disease stress and susceptibility and (b) as markers for the rate of recovery from incurred stresses to complement the development of quantifiable endpoint, nutrition-based management strategies for limiting the duration and intensity of host response to immune challenge.
- 2) Assess the development and practical short-term application of stabilization nutrient adjunct treatments involving the timely administration of antioxidant compounds, competitive anti-metabolites, or specific enzyme co-factors availability and manipulation to minimize the duration of feed-intake and growth slumps which develop in conjunction with periods of naturally-occurring but temporally predictable management and life stresses such as birth, parturition, weaning, transportation, and vaccination.
- 3) Assess the magnitude and prevalence of subpopulations of cattle that phenotypically display health profiles suggestive of prolonged and more intense adverse (hyper-responsive) metabolic response to low-level bacterial toxin challenge. Determine the extent to which detectable polymorphisms in specific inflammatory cytokine gene promoter regions correlate with the increased propensity for subpopulations of genetically related animals to hyper-respond to immune challenge with excessive inflammatory cytokine production and downstream nitro-oxidative modification of protein in response to commonly encountered stresses compared to those responses displayed by an “average” population.

East Lansing, Michigan -- Avian Disease and Oncology Research Lab

- [Genomics and Immunogenetics of Economically Important Traits of Poultry](#)

CRIS Project Title: *Genomics and Immunogenetics of Economically Important Traits of Poultry*
(NP 101-Components II, III, IV)

SY (2.60): H. Cheng, H. Zhang, Henry Hunt

Appropriated Annual Funding: \$1,366,870

Related Sibling CRIS Projects:

- 01R – Identification of Marek's disease resistance genes in chicken through virus-host protein interactions. 11/1/02 – 10/31/06. \$273,000 (USDA NRI grant).
- 02T – Verification of QTL for resistance to Marek's disease in commercial chickens. 10/1/99 – 9/30/03. \$260,000 (USDA NRI grant).
- 03S – Verification of QTL for resistance to Marek's disease in commercial chickens. 3/1/00 – 9/30/03. \$40,000 (SCA with Dr. Morris Soller, Hebrew U. of Jerusalem, Israel).
- 04T – Positional candidate genes for resistance to Marek's disease through DNA microarrays. 12/15/00 – 11/30/04. \$325,000 (USDA NRI grant).
- 05S – Positional candidate genes for resistance to Marek's disease through DNA microarrays. 4/17/01 – 9/30/03. \$45,000 (SCA with Dr. Joan Burnside, U. of Delaware).
- 06R – Bridging genome sequence to the prevention of Marek's disease in poultry. 9/15/01 – 9/14/06. \$283,561 (USDA IFAFS grant).
- 08S – Cloning a virulent Marek's disease virus. 1/23/03 – 5/30/07. \$480,184 (SCA to Dr. Jerry Dodgson, Michigan State U.).
- 09R – Antigen presentation by RFP-Y class molecules in the chicken. 12/15/01 – 12/13/04. \$18,270 (USDA NRI grant).
- 10R – High resolution microarray and protein analysis of chicken immune responses. 6/1/04 – 5/30/07. \$999,824 (USDA NRI grant).
- 11R – Use of RNAi to block viral infections in poultry. 9/15/04 – 9/14/07. \$66,000 (USDA NRI grant).
- 12S – High resolution microarray and protein analysis of chicken immune responses. 9/30/04 – 5/31/07. \$142,637 (SCA with Dr. Ming Ouyang, Informatics Institute).
- 13S – High resolution microarray and protein analysis of chicken immune responses. 9/16/04 – 5/31/07. \$499,888 (SCA with Dr. Joan Burnside, U. of Delaware).
- 14M – Investigation of epidemiology, diagnosis and control of retrovirus infections in poultry. 5/11/04 – 2/28/09. Memorandum of understanding.
- 15R – Validation and characterization of a high-density chicken SNP map. 5/15/05 – 5/14/07. \$616,803 (USDA NRI grant).
- 16G – Validation and characterization of a high-density chicken SNP map. 9/12/05 – 4/30/07. \$59,909 (SCA with Dr. Bill Muir, Purdue U.).
- 17S – Validation and characterization of a high-density chicken SNP map. 5/01/05 – 4/30/07. \$161,100 (SCA with Dr. Martien Groenen, Wageningen Agricultural U., The Netherlands).

Species Impacted: Poultry

Listing of Project Objectives:

- 1) Develop an integrated genetic, physical, and comparative map of the chicken genome based on microsatellite markers and expressed sequence tags (ESTs).
- 2) Identification and characterization of chicken quantitative trait loci (QTL)/genes that either confer resistance to MD and other viral-induced tumors, or account for reproductive or growth traits.
- 3) Develop methods based on knowledge of the chicken major histocompatibility complex (MHC) genes for optimizing antigen presentation to enhance the vaccinal response and immunity to MDV and other viral tumor-inducing pathogens.
- 4) Evaluation of methods for development of commercial chickens with resistance and lack of endogenous virus to improve the safety of live embryo vaccines and production of transgenic chickens.

St. Paul, Minnesota -- Plant Science Research Unit

- [Designing Forages with Improved Cell Wall Digestibility and Greater Intake Potential](#)

CRIS Project Title: *Designing Forages with Improved Cell Wall Digestibility and Greater Intake Potential*
(NP 101- Component V)

SY (0.65): H. Jung (Lead)

Appropriated Annual Funding: \$182,820

Related Sibling CRIS Projects: None

Species Impacted: Dairy Cattle, Beef Cattle, Sheep

Listing of Project Objectives:

- 1) Identify the cell wall and tissue structures that limit digestibility of forage cell wall polysaccharides and test genetic approaches for eliminating these limitations to forage digestibility.
- 2) Evaluate the Australian Shear Force System for predicting forage intake potential and provide a biological explanation, based on cell wall and tissue structures, for the accuracy of this intake prediction tool.

Miles City, Montana – Fort Keogh Livestock and Range Research Lab

- [Develop Beef Cattle Better Suited for Sustainable Production](#)

CRIS Project Title: *Develop Beef Cattle Better Suited for Sustainable Production*
(NP 101-Components II, III, IV, VIII)

SY (4.60): M. McNeil (Lead), A. Roberts, T. Geary, L. Alexander, E. Grings, R. Waterman

Appropriated Annual Funding: \$1,476,517

Related Sibling CRIS Projects:

5434-31000-014-01S Enhanced Sustainability of Rangeland Agriculture Through Increased Animal Productivity

5434-31000-014-02S Beef Cattle Improvement through Genetic Prediction Technology

Species Impacted: Beef Cattle

Listing of Project Objectives:

- 1) Determine the role of follicular growth in maintaining pregnancy during early gestation.
- 2) Determine genetic and environmental partitioning of phenotypic variation in reproductive traits of heifers and bulls measured early in life under two differing planes of nutrition and evaluate their relationships with lifetime reproductive performance of cows.
- 3) Identify quantitative trait loci and novel genes affecting attainment of puberty, probability of conception, return to estrus after calving, reproductive longevity, and other components of sustained reproduction.
- 4) Develop selection indexes to increase genetic potential for profitability of beef production through optimal use of feed inputs in sustainable cow-calf production.

Clay Center, Nebraska – Roman L. Hruska U.S. Meat Animal Research Center

Genetics and Breeding Research Unit

- [Germplasm Evaluation and Conservation in Beef Cattle](#)
- [Identification and Characterization of Genetic Variation for Factors Affecting Fetal Survival and Litter Size in Swine](#)
 - [Identification and Validation of Economically Important Loci in Swine](#)
 - [Genetic Mapping in Cattle Based on Single Nucleotide Polymorphisms](#)
 - [Characterization and Use of Genetic Variation in Sheep](#)
- [Characterization and Efficient Use of Resources in Beef Production Systems](#)

CRIS Project Title: *Germplasm Evaluation and Conservation in Beef Cattle* (NP 101- Components II, III, IV)

SY (2.7): L.V. Cundiff (Lead); D. VanVleck, G. Snowder

Appropriated Annual Funding: \$791,868

Related Sibling CRIS Projects: None

Species Impacted: Beef cattle

Listing of Project Objectives:

- 1) Characterize breeds representing diverse biological types for a wide spectrum of biological traits contributing to economic beef production.
- 2) Evaluate genotype-environment interactions among breeds representing diverse biological types from tropical and temperate regions of the world in subtropical and temperate regions of the U.S.
- 3) Determine favorable and unfavorable effects of a major gene for leanness (inactive myostatin resulting in muscular hypertrophy) on economically important traits in alternative mating systems.
- 4) Develop procedures and estimate genetic parameters to combine use of quantitative and molecular data into genetic evaluations for beef cattle.

CRIS Project Title: *Characterization and Use of Genetic Variation in Sheep* (NP 101- Components II, III, IV)

SY (1.3): K. Leymaster (Lead); D. VanVleck, B. Freking

Appropriated Annual Funding: \$296,245

Related Sibling CRIS Projects: None

Species Impacted: Sheep

Listing of Project Objectives:

- 1) Evaluate growth, composition, and meat quality of breeds representing an extensive range of biodiversity.
- 2) Evaluate wool and hair breeds for production efficiency under intensive and extensive management systems.
- 3) Identify QTL and exploit allelic differences for reproduction, growth, carcass composition, and meat tenderness.
- 4) Estimate genetic relationships among component traits affecting biological efficiency.

CRIS Project Title: *Identification and Characterization of Genetic Variation for Factors Affecting Fetal Survival and Litter Size in Swine*
(NP 101- Components I, II, III, IV)

SY (1.0): B. Freking (Lead); K. Leymaster

Appropriated Annual Funding: \$307,049

Related Sibling CRIS Projects:

5438-31000-069-01N: Characterizing the Expression Profile of the Prolactin Receptor Gene During Different Stages of Gestation.

5438-31000-069-03R: Association of Imprinted Genes with Reproductive Efficiency in Swine.

Species Impacted: Swine

Listing of Project Objectives:

- 1) Develop a high density porcine comparative genetic linkage map based on markers developed within expressed genes.
- 2) Develop genomic tools and reagents to identify profiles of expressed genes in tissues critical to component traits of litter size.
- 3) Evaluate direct and correlated responses and identify the underlying genomic variation exploited by selection for component traits of litter size.

CRIS Project Title: *Genetic Mapping in Cattle Based on Single Nucleotide Polymorphisms*
(NP 101- Components III, IV)

SY (1.0): R. Stone (Lead)

Appropriated Annual Funding: \$1,034,154

Related Sibling CRIS Projects:

5438-31000-070-01S: Fingerprinting and End Sequencing BAC Clones to Contribute to the International Effort of Building a Bovine and Porcine Physical (BAC) Map

Species Impacted: Beef Cattle, Swine

Listing of Project Objectives:

- 1) Develop a comparative map based on the linkage mapping of bovine ESTs associated with SNPs. This will provide a skeletal bovine SNP map which can be compared to the gene-rich human map and the human genome sequence.
- 2) Develop SNP-based markers suitable for high throughput genomic scans or for targeting specific QTL intervals. The ESTs and genes mapped under objective 1 and those mapped by radiation hybrids by other groups will be the starting resources for obtaining the desired marker coverage.
- 3) Mirror the bovine linkage map onto a BAC contig map. A bovine BAC contig map and the corresponding BAC pools and filters are being constructed through specific cooperative agreements.

CRIS Project Title: *Identification and Validation of Economically Important Loci in Swine*
(NP 101- Components II, III, IV)

SY (1.0): G. Rohrer (Lead)

Appropriated Annual Funding: \$458,436

Related Sibling CRIS Projects: None

Species Impacted: Swine

Listing of Project Objectives:

- 1) Identify chromosomal regions which affect economically important traits in swine with particular emphasis on traits affecting production efficiency and pork palatability.
- 2) Determine the importance of the identified genomic regions from Objective 1 to the pork industry by evaluating their effects on performance in commercial swine populations.
- 3) Develop genetic tests which can be used as tools to improve selection in commercial swine populations for the most important loci validated in Objective 2.
- 4) Assist the International Swine Genome Sequencing Project to assemble sequence data by determining genome position of unmapped contigs and assess single nucleotide polymorphism markers in commercial U.S. pigs.

CRIS Project Title: *Characterization and Efficient Use of Resources in Beef Production Systems*
(NP 101- Components II, VIII)

SY (2.0): T. Jenkins (Lead), C. Williams

Appropriated Annual Funding: \$409,721

Related Sibling CRIS Projects: None

Species Impacted: Beef Cattle

Listing of Project Objectives:

- 1) Quantify breed differences in specific production traits for use in decision support software and efficiency during the period from parturition to weaning of diverse biological types of cattle.
- 2) Integrate component models of nutrient intake, retention, and excretion into existing decision support software.
- 3) Reformulate decision support software to draw inferences to a broader range of mating system options and nutrient management strategies.

Clay Center, Nebraska – Roman L. Hruska U.S. Meat Animal Research Center

Meats Research Unit

- [Strategies to Optimize Carcass Yield and Meat Quality of Red Meat Animals](#)

CRIS Project Title: *Strategies to Optimize Carcass Yield and Meat Quality of Red Meat Animals*
(NP 101- Components II, III, VII)

SY (2.0): T. Wheeler (Lead), S. Shackelford, Vacant

Appropriated Annual Funding: \$886,564

Related Sibling CRIS Projects:

5438-31430-003-06T (NCBA) Prediction of Beef Carcass Cut-Out Yields Using the MARC Beef Image Analysis System

5438-31430-003-07T (NCBA) Mapping the Muscle Fiber Orientation and Adapting Slice Shear Force for Major Beef Muscle

No CRIS number (NCBA) --less than \$25,000 Standardization of Slice Shear Force Measurements across Research Institutions and Evaluation of Repeatability of Slice Shear Force Measurement at Multiple Institutions

5438-31430-003-08T (NCBA) On-Line Classification of U.S. Select Beef Carcasses for Longissimus Tenderness Using Near-Infrared Reflectance Spectroscopy

Pending (U.S.-Israel BARD) Caspase and the Calpain-Calpastatin System in Meat Tenderization

5438-31430-003-09T (NCBA) Effect of Quality Grade and Postmortem Aging Time on Top Sirloin and Shoulder Clod Tenderness

Species Impacted: Beef Cattle, Swine, Sheep

Listing of Project Objectives

- 1) Evaluate effects of breeds of cattle, swine, and sheep representing diverse biological types on carcass composition and meat quality,
- 2) Identify quantitative trait loci (QTL) and genes for carcass composition and meat quality traits in cattle, swine, and sheep and utilize proteomics/ functional genomics tools to determine their mode of action.
- 3) Use biochemical and genomics tools to test the hypothesis that the calpain proteolytic system regulates muscle growth and development.
- 4) Evaluate the efficacy of various non-invasive methods and develop new non-invasive methods to predict meat quality.
- 5) Identify the relative role of connective tissue, muscle shortening, postmortem proteolysis, and other physiological parameters (such as ionic strength, pH, conductivity, temperature) in tenderness of various muscles and use this information to develop muscle-specific intervention strategies to optimize meat tenderness.

Clay Center, Nebraska – Roman L. Hruska U.S. Meat Animal Research Center

Molecular Genetics Research Unit

- [Discovery and Use of Quantitative Trait Loci for Genetic Improvement of Beef Cattle](#)
- [Development and Application of Genomic Technologies to Improve Beef Cattle, Swine, and Sheep](#)
 - [Bioinformatics for Livestock](#)

**CRIS Project Title: *Bioinformatics for Livestock*
(NP 101- Components III, IV)**

SY (3.1): J. Keele (Lead), G. Harhay, W. Snelling, R. Wiedmann

Appropriated Annual Funding: \$1,330,826

Related Sibling CRIS Projects:

5438-31000-071-01S High Throughput Fingerprinting of BAC Clones to Develop a Bovine Physical Map

5438-31000-071-02S Sequencing Ends of BAC Clones to Develop a Bovine Physical Map

Species Impacted: Beef Cattle, Swine, Sheep

Listing of Project Objectives:

- 1) Evaluate the effectiveness of intelligent agents (autonomous computer programs) to gather and combine genomic data from several laboratories, each with different formats and nomenclatures.
- 2) Build a system that autonomously constructs comparative maps among human, cattle, swine, and sheep while integrating data from different types of maps (e.g., linkage, RH and BAC). Configure system to automatically update comparative maps when new data is available. Present the latest maps to the research community via the WWW. Develop a tool that uses similarities between livestock and human DNA sequences to direct the design of primers for livestock genetic mapping.
- 3) Incorporate the livestock linkage maps into the comparative maps. Automate linkage mapping for cattle, swine, and sheep.
- 4) Incorporate functional genomics information into comparative maps using between-species sequence similarity and controlled vocabularies. Identify cDNA clones containing full length mRNA for comprehensive sequencing. Identify different isoforms of transcripts.

**CRIS Project Title: *Discovery and Use of Quantitative Trait Loci in Beef Cattle*
(NP 101- Components II, III, IV)**

SY (3.0): G. Bennett (Lead), E. Casas, M. Thallman

Appropriated Annual Funding: \$832,107

Related Sibling CRIS Projects:

5438-31000-072-01S Development and use of a high speed genotyping system for livestock

Species Impacted: Beef Cattle

Listing of Project Objectives:

- 1) Increase the coverage and number of markers on the bovine linkage map by adding microsatellite and single nucleotide polymorphic (SNP) markers.
- 2) Further develop statistical theory and software to track inheritance in complex pedigrees and incorporate the developments in to genetic evaluation software.
- 3) Identify strategies for improving the discovery and use of QTL, haplotype, and gene-associated variation for beef cattle improvement.
- 4) Characterize variation related to previously identified QTL for carcass traits in samples of diverse germplasm from the MARC Germplasm Evaluation Project.

- 5) Discover new QTL and refine locations of previously identified QTL for cattle reproduction, and, continue to improve the application of marker-assisted selection in the cattle twinning herd.
- 6) Identify pedigree structures and make matings in experimental purebred and composite cattle populations that will further the goals of cattle genomics efforts at MARC including testing of marker-assisted selection theory, verification of QTL effects, and/or detection of genetic effects using SNP haplotype-based methods.

CRIS Project Title: *Development and Application of Genomic Technologies to Improve Beef Cattle, Swine, and Sheep*
(NP 101- Components III, IV)

SY (3.0): T. Smith (Lead), D. Nonneman, Vacant

Appropriated Annual Funding: \$861,739

Related Sibling CRIS Projects:

5438-31000-073-01R: *Functional Genomics of Well-Being and Milk Quality in Cattle*. Agreement total funding: \$97,475. FY2001-5

Species Impacted: Beef Cattle, Swine, Sheep

Listing of Project Objectives:

- 1) Develop genome-wide comparative genetic linkage maps in swine and cattle using nucleotide sequence variation as markers, and integrate with physical genome maps.
- 2) Identify specific candidate genes for meat tenderness and reproduction QTL, develop and validate marker systems to monitor these genes in research and commercial herds.
- 3) Correlate effects of observed nucleotide sequence variation in candidate genes located under QTL peaks with functional variation in the properties of the gene product by developing, as appropriate, assays to test receptor binding, cellular location of expression, enzyme turnover, transcriptional activity, protein levels, or DNA binding properties.
- 4) Produce complete cDNA sequences for predicted full length clones in the MARC bovine and porcine EST libraries and deposit these sequences in a national database.

Clay Center, Nebraska – Roman L. Hruska U.S. Meat Animal Research Center

Nutrition Research Unit

- [Strategic Feeding to Optimize Nutrient Use and Reduce Environmental Impact of Cattle and Sheep](#)
- [Strategies to Improve Efficiency of Nutrient Use and Minimize Nutrient Excretion by Swine](#)

CRIS Project Title: *Strategic Feeding to Optimize Nutrient Use and Reduce Environmental Impact of Cattle and Sheep*
(NP 101- Components I, II, III, IV, V)

SY (3.0): C. Ferrell (Lead), H. Freetly, Vacant

Appropriated Annual Funding: \$1,209,813

Related Sibling CRIS Projects: None

Species Impacted: Beef Cattle, Sheep

Listing of Project Objectives:

- 1) Quantify changes in energy expenditures and amino acid utilization of diverse biological types in response to changes in nutrient availability.
- 2) Quantify relationships between amino acid requirement and energy availability in cows and ewes.
- 3) Match energy and amino acid supply to animal needs to improve efficiency and reduce environmental impact of beef cattle.
- 4) Conduct research focused on improving the feed efficiency of cattle.

CRIS Project Title: *Strategies to Improve Efficiency of Nutrient Use and Minimize Nutrient Excretion by Swine*
(NP 101- Components III, V)

SY (2.0): J. Klindt (Lead), Vacant

Appropriated Annual Funding: \$597,848

Related Sibling CRIS Projects: None

Species Impacted: Swine

Listing of Project Objectives:

- 1) Synchronize dietary protein quantity and quality with the requirements of individual swine utilizing the metabolite, blood urea nitrogen, as an assessment of adequacy of nutriture.
- 2) Identify quantitative trait loci (QTL) for efficient utilization of dietary amino acids as indicated by assessment of amino acid deamination by measurement of blood urea nitrogen.
- 3) Develop practical nutritional strategies to reduce urinary excretion of volatilizable nitrogen, concurrently minimize fecal phosphorus excretion, and improve carcass quality through use of feedstuffs containing sufficient quantities of fermentable nonstarch polysaccharides and available phosphorus.

Clay Center, Nebraska – Roman L. Hruska U.S. Meat Animal Research Center

Reproduction Research Unit

- [Genetic and Phenotypic Predictors of Gametogenesis in Beef Bulls and Swine](#)
 - [Genetic and Phenotypic Determinants of Litter Size in Swine](#)
- [Physiological and Genomic Regulation of Ovarian Follicular Development in Beef Cattle](#)
 - [Genome Mapping of Ovulation Rate and Uterine Capacity in Swine](#)

CRIS Project Title: ***Genetic and Phenotypic Predictors of Gametogenesis in Beef Bulls and Swine***
(NP 101- Components I, II, III)

SY (3.0): J. Ford (Lead), T. Wise, Vacant

Appropriated Annual Funding: \$641,861

Related Sibling CRIS Projects:

NFCA # 58-5438-4-0112N; Genome Mapping of Ovulation Rate and Uterine Capacity in Swine

Species Impacted: Beef Cattle, Swine

Listing of Project Objectives:

- 1) Identify breed differences for testis development and semen quality in bulls representing unique germplasm for improvement in efficiency of U.S. beef production.
- 2) Use genomic markers and physiological traits to develop procedures to identify boars and bulls with the greatest potential for sperm production.
- 3) Use genomic markers and physiological traits to develop procedures to identify gilts with the greatest potential for rapid return to estrus after weaning of piglets.

CRIS Project Title: ***Genetic and Phenotypic Determinants of Litter Size in Swine***
(NP 101- Components I, II, III, IV)

SY (2.0): J. Vallet (Lead), Vacant

Appropriated Annual Funding: \$645,019

Related Sibling CRIS Projects:

5438-31000-077-01S: Proteomic Analysis in Domestic Livestock

5438-31000-077-02T: Intrauterine Folate Binding Proteins during Pregnancy in Swine

5438-31000-077-03N: Confirmation of a Genetic Marker for Litter Size in a Commercial Population of Swine

5438-31000-077-05N: Food Safety Study for Progeny of Cloned Pigs

Species Impacted: Swine

Listing of Project Objectives:

- 1) Evaluate phenotypic and genetic changes in pigs selected using either random selection, selection for ovulation rate or selection for uterine capacity.
- 2) Determine the effects of accelerating/decelerating uterine function and conceptus development using hormonal manipulation on subsequent uterine capacity and litter size.
- 3) Define promoter and enhancer elements that control the transcription of the uteroferrin gene expressed by the uterine endometrium during pregnancy and exploit that information to develop transgenic constructs capable of altering the expression in these tissues of genes that influence reproductive efficiency.
- 4) Identify the gene polymorphisms that correspond to QTLs for reproduction traits (ovulation rate, uterine capacity, fetal erythropoiesis) in swine and determine the effects of these polymorphisms on gene transcription, mRNA processing and translation, and protein function influencing each trait.

CRIS Project Title: *Physiological and Genomic Regulation of Ovarian Follicular Development in Beef Cattle*
(NP 101- Components I, II, III, IV)

SY (3.0): S. Echternkamp (Lead), M. Allan, R. Cushman

Appropriated Annual Funding: \$958,827

Related Sibling CRIS Projects:

5438-31000-079-01S Implementation of Robust Multiplex Panels of SNPs as Diagnostic Tools for the Livestock Industry

Species Impacted: Beef Cattle

Listing of Project Objectives:

- 1) Identify and map candidate genes with potential influence on reproduction and other targeted traits in beef cattle.
- 2) Use genomic, microarray and proteomic information to identify critical regulatory steps controlling bovine reproduction and to characterize molecular bases for genetic variation between Twinner and other beef cattle populations.
- 3) Develop husbandry procedures and production systems to improve reproductive traits in beef cattle with twin births.

CRIS Project Title: *Development of an Efficient System for Production of Meat-Type Pigs in the Southern United States*
(NP 101 - Components I, II, III)

SY (1.0): J. Vallet

Appropriated Annual Funding: \$148,720

Related Sibling CRIS Projects:

5438-31000-066-01G: Development of an efficient program for production of swine in the southern US

Species Impacted: Swine

Listing of Project Objectives:

- 1) A physical facility will be developed and an effective breeding and production program will be implemented to provide a basis for conducting swine production research.
- 2) Lines of swine will be selected to establish maternal and paternal lines that can perform under the environmental conditions in the Southern United States.
- 3) A production program will be developed to ensure appropriate levels of fertility and litter size.

Ithaca, New York -- U.S. Plant, Soil and Nutrition Research Center

- [Methods for Improving Ruminal Fermentation](#)

**CRIS Project Title: Alternative Mechanisms for Improving Ruminal Fermentation
(NP 101-Component V)**

SY (1.0): J. Russell (Lead)

Appropriated Annual Funding: \$293,162

Related Sibling CRIS Projects:

Ruminal Lysine Degradation 6/29/04 – 6/29/09 #1907-31000-005-02S

Methods for Improving Ruminal Fermentation 06/01/01 – 05/31/04 #1907-31000-005-01S

Genetic Regulation and Diversity of *Escherichia coli* 4/15/00 – 4/14/03 #1907-31000-005-01S

Species Impacted: Dairy Cattle

Listing of Project Objectives:

- 1) Screen ruminal bacteria bacteriocin production and evaluate the ability of these bacteriocins to modify the end-products of fermentation in vitro.
- 2) Use bacteriocin producing ruminal bacteria (e.g. *S. bovis*) to improve silage quality by producing a more rapid decrease in pH and protecting amino acids from deamination, thereby enhancing environmental quality.
- 3) Improve animal health by inhibiting toxin producing listeria and clostridia.

El Reno, Oklahoma -- Grazinglands Research Laboratory

- [Improving Stocker Production in Sustainable Grazing Systems](#)

CRIS Project Title: *Improving Stocker Production in Sustainable Grazing Systems*
(NP 101 –Components II, V, VIII)

SY (2.80): William A. Phillips (Lead), M. Brown, B. Northup, H. Mayeux, Jr.

Appropriated Annual Funding: \$1,012,170

Related Sibling CRIS Projects:

31360-004-02S Development of Integrated Livestock and Forage Production Systems (Specific cooperative agreement between ARS and Redlands Community College, El Reno, OK)

31360-004-03N Educational Opportunities Associated with Agricultural Research (non-funded cooperative agreement with Redlands Community College, El Reno, OK)

31360-004-05N Estimation of Grain and Forage Digestibility Using Internal Indigestible Markers and Evaluation of Blood Urea Nitrogen in Beef Calves (non-funded cooperative agreement with Southwestern Oklahoma State University, Weatherford, OK)

Species Impacted: Beef Cattle

Listing of Project Objectives:

- 1) Identify and document the impact of breed type and intensity of livestock management on stocker cattle performance.
- 2) Identify and mitigate factors that limit the ability of stocker calves to rapidly adapt to novel forages presented for grazing.
- 3) Determine the factors that regulate intake, digestibility and nutrient utilization of warm – and cool–season grasses by stocker cattle.
- 4) Evaluate combinations of annual and perennial cool-season grasses to extend the grazing season and decrease the cost of stocker production.

Madison, Wisconsin -- U.S. Dairy Forage Research Center

- [Identify Cell Wall Factors Limiting Digestibility and Forage Utilization in Sustainable Dairy Farming](#)
 - [Maximizing Protein Efficiency in Dairy Production](#)
- [Completing An Expert System That Will Provide Site-Specific Nutritive Values for Feeds](#)

**CRIS Project Title: *Maximizing Protein Efficiency in Dairy Production*
(NP 101-Component V)**

SY (2.12): G. Broderick (Lead), N. Martin, M. Hall

Appropriated Annual Funding: \$805,869

Related Sibling CRIS Projects:

- 3655-21000-017-02R: Improving the Efficiency of Ruminant Ammonia Nitrogen Utilization for Milk Protein Synthesis and Reducing Nitrogen Losses in Dairy Cows (USDA-NRI Grant, Completed 2004)
- 3655-21000-017: Development of the Near Infrared Reflectance Spectral Assay for Rumen Undegraded Protein in Soybean Meal (CRADA, Active)
- 3655-21000-018-07T: Silage Quality and Losses as Affected by Silo Type (CRADA, Active)
- 3655-21000-017-04T: Effectiveness of Rumen-Protected Methionine for Reducing Dietary Crude Protein and Urinary Nitrogen Excretion in Dairy Cows (CRADA, Active)

Species Impacted: Dairy cattle

Listing of Project Objectives:

- 1) Develop and test a series of methods for reducing excessive formation of non-protein N (NPN) during ensiling of legumes to improve efficiency of utilization of forage protein in lactating dairy cows.
- 2) Develop and evaluate rapid and accurate in vitro methods, based on ruminal inocula, blends of commercial enzymes with proteolytic activity mimicking ruminal microbes, and near infrared reflectance spectroscopy, for quantifying protein degradation in the rumen.
- 3) Quantify requirements of ruminal microbes for degraded protein and fermentable energy with the objective of developing practical strategies for optimizing the balance between protein degradation and ruminal protein escape to minimize losses of degraded N from the rumen.
- 4) Characterize N flux, using mass balance approaches, to determine the impact of modifying dietary protein levels and sources, manure storage and handling, and crop rotation and feed purchases on the ability to reduce importation onto dairy farms of fertilizer and feed N and to reduce N losses to the environment.

**CRIS Project Title: *Completing an Expert System That Will Provide Site-Specific Nutritive Values for Feeds*
(NP 101-Components V, VIII)**

SY (2.12): D. Mertens (Lead), R. Muck, N. Martin, G. Broderick

Appropriated Annual Funding: \$815,875

Related Sibling CRIS Projects:

- 3655-31000-018-01S: Rapid Analysis of Wet Forages to Improve Calibrations of Dry Matter Intake and Digestion Kinetics (SCA, Completed 2004)
- 3655-31000-018-02S: Developing and Analyzing a Database of Weather and Soil Information to be Related to Forage Quality (SCA, Completed 2004)
- 3655-31000-018-03T: Direct Measurement of peNDF to Improve Dairy Rations Containing Minimum Fiber (CRADA, Active)

3655-31000-018-04T: Influence of Plant Growth Environment on the Nutritive Value of Feeds (CRADA, Completed 2004)

3655-31000-018-05R: The Effect of Lactic Acid Bacteria Silage Inoculants on the Ruminal Ecosystems, Fiber Digestibility, and Animal Performance (BARD, Completed 2003)

3655-31000-018-06S: Impact of Alfalfa Hay NDF Content and Digestibility on Dairy Cow Performance (SCA, Active)

3655-31000-018-07T: Silage Quality and Losses as Affected by Silo Type (CRADA, Active)

3655-31000-018-08S: The Nutritive Value of Legumes when Grown in a Mixture with Grasses (SCA, Active)

58-3655-4-402: Comparison of Additives to Enhance Silage Quality and Stability (Trust Agreement, Completed 2005)

Species Impacted: Dairy cattle

Listing of Project Objectives:

- 1) Develop a comprehensive and internally consistent reference database of chemical and digestion kinetic characteristics, physical properties, and biological measurements that will be used by the feed information expert system to estimate site-specific nutritive value of feeds.
- 2) Develop quantitative relationships between crop growing conditions and characteristics of feeds that can be implemented in the feed information expert system to modify nutritive value for a specific crop grown in a specific environment.
- 3) Improve the relationships used by the expert system to make site-specific adjustments in nutritive value in response to changes in nitrogen and carbohydrate fractions during harvesting, storage and preservation.
- 4) Investigate the effects of lignification and ruminal pH on digestible NDF concentration and incorporate improved predictions of digestible NDF into the expert system's estimation of energy values (TDN).
- 5) Evaluate the feed information expert system by determining sensitivity to inputs and comparing new site-specific nutritive values to current estimates, and identify relationships among laboratory analyses; crop growing, harvesting, and storage conditions; and the type of animals and their ration characteristics that need to be improved.

CRIS Project Title: *Identify Cell Wall Factors Limiting Digestibility and Forage Utilization in Sustainable Dairy Farming*
(NP 101-Component V)

SY (2.12): J. Ralph (Lead), R. Hatfield, N. Martin

Appropriated Annual Funding: \$753,037

Species Impacted: Dairy Cattle

Related Sibling CRIS:

- 3655-21000-028-08T: Elucidation of the Pathway for Natural Lignin Acylation (USDA-NRI Grant, Completed 2002)
- 3655-21000-028-10T: Altering Lignin Structure for Improved Fiber Utilization (CRADA, Completed 2002)
- 3655-21000-033-01T: Increasing Pectic Carbohydrates in Alfalfa Lines to Enhance Animal Performance (CRADA, Completed 2004)
- 3655-21000-033-02R: What is the Extent of Metabolic Plasticity in the Lignification Process, and Can It be Exploited (DOE Grant, Completed 2004)
- 3655-21000-033-03T: Elucidation of the Pathway for Natural Lignin Acylation (USDA-NRI Grant, Completed 2003)
- 3655-21000-033-04T: Altering Lignin Structure for Improved Fiber Utilization (renewal) (CRADA, Active)
- 3655-21000-033-05S: Lignin Methods (SCA, Active)
- 3655-21000-033-06R: Non-Degradative Dissolution of Wood Fiber: Basis for New and Improved Analytical Methods (USDA-NRI Grant, Active)
- 3655-21000-033-07R: What is the Extent of Metabolic Plasticity in the Lignification Process, and Can It be Exploited (renewal) (DOE Grant, Active)
- 3655-21000-033-08R: Hydroxycinnamates in Cereal Grains (USDA-NRI Grant, Active)
- 3655-21000-033-09S: Cell Wall Cross-linking by Hydroxycinnamates in Cereal Grains (SCA, Active)
- 3655-21000-033-10R: *p*-Coumarylation of Grass Lignins (USDA NRI Grant, Active)

Species Impacted: Dairy cattle

Listing of Project Objectives:

- 1) Characterize plants with genetically modified lignin-pathways in order to develop novel approaches for improving forage utilization and provide insight into the complex effects of lignin composition on cell wall digestibility and the consequent formation of indigestible residues. Delineate the role of cell wall cross-linking (the attachment of polysaccharides to other polysaccharides and to indigestible lignins) to elucidate their impact on digestibility. Develop analytical tools to answer cell wall structural questions.
- 2) Determine the characteristics of indigestible residues in order to determine what regulates their formation by dairy cows and to reveal strategies for decreasing their negative impact. c) Determine the extrinsic role of rumen environment, such as pH, specific microbial populations, and their dynamic interactions, on the digestion kinetics of cell wall components in vitro and in vivo. d) Use genetic selection to select cell wall characteristics that will improve the rate and extent of wall carbohydrate degradation.

NATIONAL PROGRAM 105 – ANIMAL WELL-BEING AND STRESS CONTROL SYSTEMS

West Lafayette, Indiana -- Livestock Behavior Research Unit

- [Ethology of Food Producing Animals](#)

CRIS Project Title: *Ethology of Food Producing Animals* (NP 105)

SY (3.28): D. Lay (Lead), S. Eicher, H. Cheng, J. Forde

Appropriated Annual Funding: \$919,504

Related Sibling CRIS Projects:

3602-32000-006-18R: *Reproduction II* as a new strategy for molting in laying hens: stress indicators, alternative method for molting, and practical implication, 10-1-05 to 9-30-08, \$314, 275

Species Impacted: Swine, Dairy Cattle, Poultry

Listing of Project Objectives:

- 1) Identify a method of molting hens that causes minimal stress and a lack of or minimum feelings of hunger.
- 2) Determine early indicators of lameness and to test the feasibility of housing changes to improve lameness incidence and severity in swine and dairy cattle.
- 3) Determine an objective method of measuring hunger in swine.
- 4) To develop a relative ranking of animal well-being of sows in gestation stalls compared to pens, based on three factors: physical, physiological, and mental state.

Starkville, Mississippi -- Poultry Research Unit

- [Nutritional and Environmental Management to Reduce Stress, Improve Quality and Improve the Efficiency of Poultry Production](#)

CRIS Project Title: *Nutritional and Environmental Management to Reduce Stress, Improve Quality, and Improve the Efficiency of Poultry Production*
(NP 105)

SY (3.70): W. Rouse (lead), W. Dozier, H. Olanrewaju, J. Purswell, S. Branton

Appropriated Annual Funding: \$1,928,664

Related Sibling CRIS Projects:

58-6406-9-0017 - Environmental Control for Broiler Well-Being and Waste Management

58-6406-2-0024 - Improving Broiler House Environment by Reducing Ammonia and Mortality

58-6406-4-0093 – Reducing Ammonia Levels in Broiler Houses

Species Impacted: Poultry

Listing of Project Objectives:

- 1) Optimize production efficiency and meat yield while minimizing stress, metabolic mortality and skeletal disorders of modern broiler chickens by the assessment of environmental parameters, feeding programs and their interactions.
- 2) Reduction of ammonia production and nitrogen excretion by dietary manipulation as broilers approach market weight while optimizing production efficiency.
- 3) Evaluation of the interactive effects of ammonia and light intensity on the health, well-being of broiler chickens.

Clay Center, Nebraska – Roman L. Hruska U.S. Meat Animal Research Center (Biological Engineering Research Unit)

- [Bioenergetic Criteria for Meat Animal Environmental Management](#)

CRIS Project Title: *Bioenergetic Criteria for Meat Animal Environmental Management*
(NP 105)

SY (1.8): John A. Nienaber (Lead), T Brown-Brandl, R. Eigenberg

Appropriated Annual Funding: \$490,840

Related Sibling CRIS Projects: None

Species Impacted: Beef Cattle, Dairy Cattle, Swine, Sheep

Listing of Project Objectives:

- 1) For growing meat animals, measure and evaluate the dynamic interactions of energetic (thermoregulation and nutritional), physiological (endocrine), and behavioral (activity and preference) responses to stressor (thermal and physical) thresholds causing reduced efficiency and performance.
- 2) Develop or improve functional relationships of performance and physiological responses to thermal environmental conditions.
- 3) Modify current livestock production system models to more fully incorporate the impact of environmental stressors on expected performance as a basis for decision support systems.

Lubbock, Texas -- Cropping Systems Research Laboratory (Livestock Issues Research Unit)

- [Development and Interpretation of Animal Well-Being Indicators to Assess Management Systems](#)

CRIS Project Title: *Development and Interpretation of Animal Well-Being Indicators to Assess Management Systems*
(NP 105)

SY (1.5): Jeff Carroll (Lead), S. Dowd, Vacant

Appropriated Annual Funding: The NTL for the Animal Physiology Research Unit in Columbia, Missouri was \$580,515.00. This amount from project # 3622-32000-005 was transferred to the Livestock Issues Research Unit in Lubbock, Texas and placed it in project # 6208-32000-003 resulting in a NTL for the Livestock Issues Research Unit in Lubbock of \$582,009.00.

Related Sibling CRIS Projects: None

Species Impacted: Beef Cattle and Swine

Listing of Project Objectives

- 1) Determine the mechanisms by which stressors affect performance, health, and well-being of livestock from birth to market, and how such mechanisms are influenced by endogenous and exogenous factors.
- 2) Evaluate responses of individual animals to various managerial and environmental stressors, and develop alternative production practices and systems.
- 3) Evaluate the feasibility of using applied animal behavioral responses for early detection of homeland security threats.

APPENDIX 3 – ANNUAL REPORT INFORMATION (2001-2004)

National Program 101: Food Animal Production

National Program Annual Report Introductions Fiscal Years 2001 – 2004

2001: A joint National Program Workshop for the Animal Genomes, Germplasm, Reproduction, and Development Program (NP101) and the Animal Production Systems Program (NP102) was held at the Holiday Inn, College Park, Maryland, on February 13, 2000. An outcome of the workshop was the merging of the two National Programs to integrate the sciences with the structure and renaming the combined National Program Food Animal Production (NP101). Prospectuses and project plans were developed for 40 projects during 2001. The peer panels will meet early in 2002 to review the Food Animal Production National Program.

Animal Production, Product Value, and Safety is indebted to Drs. Chadwick Chase, Claud (Rick) Barb, Michael MacNeil, Curtis Van Tassell, and John McMurtry for serving details as Acting National Program Leader (NPL), Food Animal Production.

The Program at Range and Livestock Research Unit was reviewed June 25 to 27, 2001.

An increase of \$450,000 for Fiscal Year (FY) 2001 was appropriated for genomics research.

ARS cohosted the Stakeholders Workshop for Animal Agriculture: FAIR 2002 Implementation Partnerships, November 27, to 29, 2001 at the Holiday Inn, College Park, Maryland.

Several scientists were recipients of national research awards, including:

- H. Duane Norman, from the Animal Improvements Program Laboratory, Animal and Natural Resources Institute, Beltsville, Maryland, was awarded the American Dairy Science Association 2001 Fellow Award.
- J. Joe Ford from the U.S. Meat Animal Research Center, Clay Center, Nebraska, was awarded the American Society of Animal Science Animal Physiology and Endocrinology Award.
- Gary L. Bennett from the U.S. Meat Animal Research Center, Clay Center, Nebraska, was awarded the American Society of Animal Science Rockefeller Prentice Award in Animal Breeding and Genetics.

2002: Approximately 40 project plans contributing to the Food Animal Production National Program were approved by peer panel review in 2002. The project plans contain objectives to address problems consistent with the mission of ARS and approaches that ensure the objectives will be met on a timely basis. Stakeholders, customers and partners contributed to defining these problem areas. Meeting the objectives contribute to the effective conversion of resources to food animal products while addressing societal concerns for the environment. Meeting this challenge provides solutions that ensure an ample supply of food animal products and contribute to the economic well being of the food animal producers. The National Program Staff is indebted to Drs. Curt Van Tassell, John McMurtry and Thomas Jenkins for serving as the Acting National Program Leader for Food Animal Production during 2002.

An increase of \$600,000 was appropriated for research to discover, test, and implement improved genetic evaluation techniques for economically important traits in dairy cattle.

An increase of \$400,000 was appropriated for research on evaluation, collection, and storage of animal germplasm.

An increase of \$720,000 was appropriated for research on improving efficiency of cloning.

An increase of \$200,000 was appropriated for research on Coccidiosis.

ARS co-hosted the Stakeholders Workshop for Animal Agriculture: FAIR?2002 Implementation Partnerships, November 27, to 29, 2001 at the Holiday Inn, College Park, MD.

Several scientists were recipients of national research honors, some of which include:

Thomas Geary, from Ft. Keogh Livestock and Animal and Range Laboratory in Miles City, Montana, was awarded the Western Section of American Society of Animal Science 2002 Young Scientist of the Year Award.

Tommy L. Wheeler from the U.S. Meat Animal Research Center in Clay Center, Nebraska, was awarded the American Society of Animal Science Meats Research Award.

Paul M. Van Raden from the Animal Improvement Programs Laboratory, Animal and Natural Resources Institute, Beltsville, MD was awarded the National Association of Animal Breeders' Research Award.

Lawrence A. Johnson, retired Research Leader at Germplasm and Gamete Physiology Laboratory, Animal Natural Resources Institute, Beltsville, MD, was named as a Fellow by the American Society of Animal Science.

2003: The food animal production national program is charged with conducting cutting edge research to contribute to increased efficiency and sustainability of production of beef and dairy cattle, poultry, swine, and sheep. Research efforts in the animal sciences over the past century have had dramatic impacts on animal agriculture both in terms of improved biological and economic efficiency of production and in terms of quantity, quality, and safety of animal products. Many major challenges remain, however, requiring the dedicated focus of long-term research teams, particularly in the areas of reproductive longevity and well-being, product quality, reduction of feed and energy inputs, enhancements in nutrient retention, and reduction of negative environmental impacts.

The program experienced a high level of productivity and success in 2003. In total, 92 scientists working at 17 locations across the U.S. were engaged in over 50 research projects in the program. Research projects in this program area were approved through the ARS Office of Scientific Quality Review in 2002, making this the first year of implementation of these five-year project efforts.

During the past year, program increases were appropriated for forage animal pasture research (\$800,000 to Lexington, KY), bovine genetics (\$300,000 to Beltsville, MD), bioinformatics (\$350,000 to Beltsville, MD and \$250,000 to Clay Center, NE), and dairy forage research (\$1,000,000 to Madison, WI), bringing the total appropriations in the national program to over \$42M.

A number of new scientists were welcomed to the program during 2003 including Steven Trabue (Ames, IA); Brent Woodward (Dubois, ID); Jim Strickland and Glen Aiken (Lexington, KY); Phil Purdy (Fort Collins, CO); Mark Allan (Clay Center, NE); and Melvin Kuhn (Beltsville, MD).

USDA/ARS was pleased to name Ronnie Green as the national program leader for food animal production in February of 2003 and looks forward to his leadership. Special appreciation is extended to a number of individuals who served in detail in this position during the search for a new national program leader including John McMurtry and Curt Van Tassell (Beltsville, MD), Mike MacNeil (Miles City, MT), Chad Chase (Brooksville, FL) and Tom Jenkins (Clay Center, NE). Their contributions, coupled with those of national program leaders Lewis Smith and Evert Byington were invaluable.

Several scientists in the national program were recognized with prominent awards for their research including:

Curt Van Tassell, Beltsville, MD, Herbert L. Rothbart Outstanding Early Career Scientist, USDA/ARS

Curt Van Tassell, Beltsville, MD, 2003 Young Research Scientist of the Year, Northeast Section of the American Dairy Science Association

Eduardo Casas, Clay Center, NE, Early Career Scientist, Northern Plains Area, USDA/ARS

John Klindt, Clay Center, NE, National Pork Board Swine Innovation Award

Cal Ferrell, Clay Center, NE, President, Midwest Section of American Society of Animal Science

Tim Smith, Clay Center, NE, Outstanding Young Scientist, Midwest Section of American Society of Animal Science

Ron Christenson, Clay Center, NE, Animal Physiology and Endocrinology Award, American Society of Animal Science

Brad Freking and Kreg Leymaster, Clay Center, NE, Superior Performance Award, Northern Plains Area, USDA/ARS

Darrell Light, Clay Center, NE, Outstanding Technical Support Award, Northern Plains Area, USDA/ARS

Elaine Grings, Miles City, MT, Outstanding Achievement Award, Society for Range Management

Dave Mertens, Madison, WI, American Feed Industry Association Award, American Dairy Science Association

Brian Kerr, Ames, IA, Superior Performance Award, Midwest Area, USDA/ARS

The high quality and impact of research conducted in the program was further evidenced by the fact that scientists delivered over 90 invited presentations at international symposia during the past year. Three CRADAs were instituted and two patents were filed by researchers in the program. Additionally, a number of extramural grant awards were received by scientists in the program, many supported by cooperative research programs with partners at land grant universities. Administrator's Postdoctoral Awards were granted to Hans Cheng (East Lansing, MI) and Mark Richards (Beltsville, MD).

A number of meetings and workshops were sponsored by this national program in the past year including Livestock Genomes: Bioinformatics and Annotation Challenges (Conroe, TX); Chicken Genome: Outlook and Applications (Atlanta, GA); ARS Swine Production Research Workshop (Ames, IA); ARS Poultry Production Workshop (Orlando, FL); and DISCOVER Conference on Antibiotic Use in Animal Agriculture (Abe Lincoln Lodge, IN).

This is a particularly exciting time for this research and program area. In the coming year, draft genome sequences will be released for the chicken and the cow. Efforts are now underway to do the same for the swine genome in 2005. Major efforts are being put in to place to build infrastructure in the agency for bioinformatics, functional genomics, and proteomics. These efforts have required creative approaches to funding and the leveraging of resources across federal agencies, international governments, and private industry. These developments open a new frontier for research in food animal production and coincidentally have elevated the expectations of the future impact of this program.

The following sections of the report summarize high impact research results addressing the eight objectives in the current national program action plan.

2004: The food animal production national program is charged with conducting cutting edge research to contribute to increased efficiency and sustainability of production of beef and dairy cattle, poultry, swine, and sheep. Research efforts in the animal sciences over the past century have had dramatic impacts on animal agriculture both in terms of improved biological and economic efficiency of production and in terms of quantity, quality, and safety of animal products. Many major challenges remain, however, requiring the dedicated focus of long-term research teams, particularly in the areas of reproductive longevity and well-being, product quality, reduction of feed and energy inputs, enhancements in nutrient retention, and reduction of negative environmental impacts.

The program again experienced a high level of productivity and success in 2004. In total, 92 full-time scientists working at 17 locations across the U.S. were actively engaged in 50 research projects in the program. Research projects in this program area were approved through the ARS Office of Scientific Quality Review in 2002, making this the second year of implementation of these five-year project efforts.

During the past year, program increases were appropriated for forage animal pasture research (\$536,814 to Lexington, KY), bovine genetics (\$536,814 to Beltsville, MD), bioinformatics (\$223,673 to Clay Center, NE), dairy forage research (\$1,252,566 to Madison, WI), beef cattle feed efficiency genomics (\$270,000 to Clay Center, NE),

and grazing beef systems (\$89,469 to Beaver, WV) bringing the total appropriations in the national program to over \$39M.

A total of eight new permanent scientists were welcomed to the program during 2004 including: Dr. Lee Alexander (Miles City, MT); Dr. LeAnn Blomberg (Beltsville, MD); Dr. John B. Cole (Beltsville, MD); Dr. Randy Dinkins (Lexington, KY); Dr. Mary Beth Hall (Madison, WI); Dr. Isabelle Kagan (Lexington, KY); Dr. Heathcliffe Riday (Madison, WI); and Dr. Richard Waterman (Miles City, MT).

Ronnie Green served as the national program leader for the program in 2004 with invaluable co-lead responsibilities provided by Lewis Smith. Contributions to the national program were also provided by program team members Evert Byington, Cyril Gay, and Robert Heckert.

Several scientists in the national program were recognized with prominent awards, including:

Curt Van Tassell, Beltsville, MD, Presidential Early Career Award for Scientist and Engineers

Curt Van Tassell, Beltsville, MD, 2004 Outstanding Young Scientist, American Dairy Science Association

Duane Norman, Beltsville, MD, Outstanding Alumni, College of Agricultural Science, Pennsylvania State University

Chad Chase, Brooksville, FL, 2004 Researcher of the Year, Florida Cattlemens Association

Larry Cundiff (as a part of team with Keith Gregory and Bob Koch), Clay Center, NE, Beef Industry Top 40 Award, BEEF Magazine 40th Anniversary

Keith Gregory (retired), Clay Center, NE, USDA/ARS 2004 Hall of Fame Inductee

Tom Jenkins, Clay Center, NE, 2004 Pioneer Award, US Beef Improvement Federation

Steve Kappes, Clay Center, NE, 2004 Continuing Service Award, US Beef Improvement Federation

Mohammad Koohmaraie, Tommy Wheeler, Steven Shackelford, Clay Center, NE, 2004 Technology Transfer Award, USDA/ARS

Dale Van Vleck, Clay Center and Lincoln, NE, 2004 A. B. Chapman Distinguished Lecturer Award, University of Wisconsin, Madison.

Dave Mertens, Madison, WI, 2004 Pioneer Hi-Bred Forage Award, American Dairy Science Association

Ronnie Green, National Program Staff, Brother of the Century Award, 100th Anniversary, National Alpha Gamma Rho Fraternity and 2003 Continuing Service Award, US Beef Improvement Federation

The high quality and impact of research conducted in the program was further evidenced by the fact that scientists delivered 56 invited presentations at international symposia during the past year. During the year, one new CRADA was established and three new patents were filed by researchers in the program. Additionally, a total of \$2.4 M in extramural grant award funding was received by scientists in the program, supported in many cases by cooperative research programs with partners at land grant universities. Administrator's Postdoctoral Awards were granted to Julie Long (Beltsville, MD), Mike MacNeil (Miles City, MT), and Harvey Blackburn and Phil Purdy (Fort Collins, CO).

Partnerships with land grant universities continued to be very important to the success of the national program in 2004. These partnerships are greatly appreciated and take on a variety of forms including work with those in the states of Arkansas, Colorado, Florida, Georgia, Idaho, Illinois, Iowa, Kentucky, Maryland, Michigan, Minnesota, Montana, Nebraska, New York, Virginia, West Virginia, and Wisconsin.

A number of meetings and workshops were sponsored by this national program in the past year including: Chicken Genome: Outlook and Applications (Atlanta, GA); USDA/ARS Swine Production Research Workshop (Ames, IA);

Beyond the Chicken Genome (Kansas City, MO); American Society of Animal Science Symposium (St. Louis, MO); USDA/ARS Forage Animal Pasture Research Unit Stakeholder Workshop (Lexington, KY); USDA Animal Genomics Workshop (Washington, DC); and ADSA DISCOVER Conference on Animal Germ Plasm (Cheyenne, WY).

This is a particularly exciting time for this research and program area. In 2004, draft genome sequences were released for the chicken (6.3-fold coverage) and the cow (initial 3.3-fold coverage). Success in obtaining the needed funding for sequencing of the swine genome was announced as this report was being written with the formal launch of the project expected to occur in the latter part of 2005. Major long-term efforts are now yielding results in building infrastructure in the agency for bioinformatics, functional genomics, and proteomics. These efforts have required creative approaches to funding and the leveraging of resources across federal agencies, international governments, and private industry. These developments open a new frontier for research in food animal production and coincidentally have elevated the expectations of the future impact of this program.

In September of 2004, USDA conducted a workshop in Washington, DC focused on needs in animal genomics. Forty-five leading researchers and administrators were invited to the workshop to formulate priority needs for agricultural animal genomics programs in the “post-sequencing” era. ARS was well represented in this group by 15 of the leading scientists from this national program. Input from this workshop is currently being utilized to develop a long-term strategic plan for USDA animal genomics research efforts. The strategic plan development is targeted for completion in the latter half of 2005 and is being lead by Ronnie Green from ARS and Muquarrab Qureshi from CSREES.

National Program 105: Animal Well-Being & Stress Control Systems

National Program Annual Report Introductions Fiscal Years 2001 – 2004

2001: Animal wellbeing is increasingly an important issue concerning animal production, and a popular theme of press articles and scientific conferences. These concerns cover issues relating to farm animal handling and management, establishing scientific measures of wellbeing, and a perceived lack of attention to the wellbeing of production animals. Animal care and feeding practices are issues being debated as extended practices in Codex Alimentarios as part of the World Trade Organization.

The Encyclopedia of Farm Animal Behavior (EFAB) is online and continually updated through the efforts of scientists in this National Program (www.liru.asft.ttu.edu/efab/index.htm).

Scientists in NP 105 participated in the annual meetings of research projects nationally coordinated by the Cooperative State Research Education, and Extension Service (CSREES). Participating in these meetings strengthens this NP by interacting with the land grant research community.

Scientists in this NP have been involved nationally and internationally representing their scientific expertise in collaborations, committees, consultations, professional and commodity organizations, and technology transfer over the past year. Some of these activities include working with the National Pork Board's Animal Welfare Committee and the National Pork Board's Swine Welfare Indexing Advisory Group; serving on Federation of Animal Science Societies (FASS) sponsored activities addressing farm animal care and handling training and communication issues and providing assistance on farm animal specialty for the American Registry of Professional Animal Scientists' committee for certification of animal workers. Several scientists received invitations to speak at international and national meetings on animal wellbeing, and contributed articles to popular farm and agricultural press.

Three projects will be peer reviewed in 2002.

2002: Animal well-being is increasingly an important issue concerning animal production, and a popular theme of press articles and scientific conferences. These concerns cover issues relating to farm animal handling and management, establishing scientific measures of well-being, and a perceived lack of attention to the well-being of production animals. Animal care and feeding practices are issues being debated as extended practices in Codex Alimentarios as part of the World Trade Organization. The Encyclopedia of Farm Animal Behavior (EFAB) is online and continually updated through the efforts of scientists in this National Program (www.liru.asft.ttu.edu/efab/index.htm).

Scientists in National Program for Animal Well-Being (NP105) participated in the annual meetings of research projects nationally coordinated by the Cooperative State Research Education, and Extension Service (CSREES) and the National Pork Board. Participating in these meetings strengthens this national program by interacting with the land grant research community, non-governmental organizations and the industry.

Scientists have been involved nationally and internationally representing their scientific expertise in collaborations, committees, consultations, professional and commodity organizations, and technology transfer over the past year. Some of these activities include working with the National Pork Board's Animal Welfare Committee and the National Pork Board's Swine Welfare Indexing Advisory Group; serving on Federation of Animal Science Societies (FASS) sponsored activities addressing farm animal care and handling training and communication issues; and providing assistance on farm animal specialty for the American Registry of Professional Animal Scientists' committee for certification of animal workers.

The Livestock Behavior Research Unit, West Lafayette, IN, has built an interdisciplinary team of scientists to address complex questions about animal well-being across three farm species; dairy, swine, and poultry. Research projects have been completed on well-being issues in all three species encompassing transportation, genetics, and management. Scientific evidence of the impact of tail-docking to the animal's well-being has been provided to several popular press publications. Members of the unit have been involved in addressing well-being issues of the

poultry industry. The Unit's scientists are now involved in a collaborative project with Purdue scientists to address well-being concerns in swine production. Multiple disciplines are incorporated into all research conducted and collaborative linkages have built teams with scientists at Purdue University, University of Florida, Iowa State University, Mississippi State University, West Virginia University, University of Tennessee, and with other ARS units (National Animal Disease Center in Ames, IA, Southeast Poultry Research Laboratory in Athens, GA, U. S. Dairy-Forage Research Center in Madison, WI, and Animal Physiology Research Unit in Beltsville, MD) to incorporate genetics, nutrition, and production to strengthen the approach to well-being questions. This is an essential requirement to the success of well-being research.

Several scientists received invitations to speak at international and national meetings on animal well-being, and contributed articles to popular farm and agricultural press. Three projects were peer reviewed in FY2002. The scientific excellence of research conducted by scientist in this national program was recognized with awards from several professional organizations.

Tami Brown-Brandl was presented the Scientific Award in Animal Biometeorology by the International Society of Biometeorology for her research paper entitled, "Thermoregulatory profile of a newer genetic line of pigs" and appears in the *Journal of Livestock Production Science*, 71:253-260.

Jeff Carroll received recognition for his research on dexamethasone and fish oil to improve the well-being of baby pigs. This research, to reduce the time to market, was recognized by the 2002 Pork Board Award. This is the third year out of four that his research was recognized. The 2002 Omega Protein Innovative Research Award, given at the Joint Animal Sciences Societies meetings in Quebec City was for his research on fish oil and immune function.

2003: Animal well-being and animal care are increasingly important issues concerning animal production and consumers. The food animal production industries and food industries in the United States have jointly worked to establish standards for animal management within the different classes of farm animals. A global conference on animal welfare is planned for February 23 to 25, 2004 as an OIE initiative bringing together science, ethical and cultural values, and practical realities to provide international guidance and standards.

Scientists in National Program for Animal Well-Being (NP105) participated in the annual meetings of research projects nationally coordinated by the Cooperative State Research Education, and Extension Service (CSREES) and other national workshops and conferences. Participating in these meetings strengthens this national program by interacting with the land grant community, non-governmental organizations and the animal industry. The Livestock Behavior Research Unit, West Lafayette, Indiana and Purdue University partners highlighted their research at the Future Trends in Animal Agriculture Round Table Meeting, in May 2003, Washington, D.C

Scientists have made nineteen invitational scientific presentations over the past year, an indicator of the relevance of their research. Scientists of the Livestock Behavior Research Unit are complimented for their grant awards as PI or CO PI totaling \$1.7 million.

The Animal Welfare Information Center, National Agricultural Library, supported the Animal Well-Being National Program with the publications:

1. Smith, C. **Information Resources on Swine Housing, Care, and Welfare**, *AWIC Resource Series No. 21*. May 2003. The document includes training materials, books and proceedings, website, and a bibliography. (<http://www.nal.usda.gov/awic/pubs/swinehousing/swinehousing2.htm>)

2. Reynnells, R., (ed) ***Proceeding: Symposium on Swine Housing and Well-being.***

1. *Stockmanship and training*; 2. *Practical Sow Housing System Design*; 3. *Consumer Perspectives*. May 2003

Note: P.O.R.K. Academy 2002. Held June 5, 2002 in Des Moines, Iowa. (On the AWIC website: <http://www.nal.usda.gov/awic/pubs/swineproceedings2002.pdf>)

3. Allen, T. **Information Resources on Induces Molting in Chickens. 1902-2002 AWIC Resource Series # 14.** September 2002. Includes CRIS reports on research, a review of the literature and books in the NAL collection. (<http://www.nal.usda.gov/awic/pubs/molting/molting2.htm>)

4. Erickson, H. **Information Resources on Fish Welfare. 1970-2003.** *AWIC Resource Series No. 20.* July 2003. Includes 12 reprinted articles on the topic; a review of the literature; CRIS reports; and professional societies, and groups (<http://www.nal.usda.gov/awic/pubs/Fishwelfare/fishwelfare.htm>)

5. Goodman, G. **Information Resources on Care and Use of Molluscs.** *AWIC Resource Series No. 22.* May 2003. Includes bibliographic information on Laboratory care and research, Aquaculture related resources and WWW resources. (<http://www.nal.usda.gov/awic/pubs/molluscs/molluscs2.htm>)

2004: Animal welfare and animal care continue to be important issues concerning animal production and consumers. A number of food industries in the United States require their suppliers to have production standards and certification.

Scientists in National Program for Animal Well-Being (NP105) participated in the annual meetings of research projects coordinated by the Cooperative State Research Education, and Extension Service and other national workshop, open houses, and conferences.

Scientists made twelve invitational scientific presentations over the past year and fifteen interviews or press articles, an indication of the relevance and quality of their research. Scientist of the Livestock Behavior Research Unit, West Lafayette, IN are complimented again for their grant awards totaling \$190,440 in FY2004.

Welcome to two new permanent full-time scientists:

William A. Dozier, III, Research Nutritionist, Poultry Research Unit, Mississippi State, MS
Hammed Olanrewaju, Research Physiologist, Poultry Research Unit, Mississippi State, MS

One patent is being pursued.

The project on animal stress at Columbia, MO was reprogrammed and relocated to Lubbock, TX.

The Animal Welfare Information Center, National Aquaculture Library, supported the Animal Well Being and Stress Control National Program with the publications:

1. Crawford, R.; D'Anna Jensen; Tim Allen; H. Erickson. **Housing, Husbandry, Care & Welfare of Selected Birds (Quail, Pheasant, Finches, Ostrich, Dove, Parrot & Others).** *AWIC Resource Series No. 26* - February 2004. URL: <http://www.nal.usda.gov/awic/pubs/Birds/birds.htm>

2. Smith, C.P. **Information Resources on the Care and Welfare of Beef Cattle.** *AWIC Resource Series No. 24.* June 2004. URL: <http://www.nal.usda.gov/awic/pubs/Beef/beef.htm>

3. Larson, J. J. Swanson, D. Berry, C. Smith and the Minor Breeds Conservancy. *Selected Reading on the History and Use of Old Livestock Breeds.* September 1991. .was converted to HTML due to the interest in the special characteristics and behaviors of some of the old breeds of livestock.
URL: <http://www.nal.usda.gov/awic/pubs/minorbreeds.htm>

4. Reynnells, R. (Ed.) **Science and Ethics Behind Animal Well-Being Assessment.** *One of a Series of Education Programs, Future Trends in Animal Agriculture.* Note: A conference held in Washington DC May 28, 2003. URL: www.nal.usda.gov/awic/pubs/ftaaproceedings/scienceandethics.pdf.

5. Rice, S. and K. Adams. **A Brief Information Resource on Assistance Animals for the Disabled.** Updated April 2004. It deals with a variety of animals including horses, dogs, and primates. A web- only document at: URL: <http://www.nal.usda.gov/awic/companimals/assist.htm>